Appendix B
Mode of Action: Inhibition of Acetylcholinesterase (AChE)

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1.0 Introduction

Chlorpyrifos, like other organophosphates, binds to and phosphorylates the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems (USEPA, 1999), leading to accumulation of acetylcholine and, ultimately, to clinical signs of toxicity. This mode of action, in which AChE inhibition leads to neurotoxicity, has been well described (Mileson et al. 1999). In 2000, the Agency concluded that inhibition of ChE was the most sensitive effect in all of the animal species evaluated and in humans, regardless of exposure duration. For the current analysis, the Agency has reviewed the studies submitted for registration as well as searched the public literature for studies in which pregnant animals and/or juvenile animals were exposed to chlorpyrifos. This review is summarized in the text and tables below. ChE inhibition is most commonly reported for the blood (plasma and RBC) and brain (whole or subsections), although a few studies have evaluated inhibition in peripheral tissues such as the heart or lung. Data in non-pregnant adults are provided as context or as supplementary information when data in the young or in pregnant animals are not available. This chapter includes 1) comparison of ChE inhibition in pregnant vs. non-pregnant adult animals, 2) examination of time course data, specifically the time to peak inhibition, and 3) discussion on how pre- or postnatal exposure, age/stage of development, duration of exposure, and method of administration may have an impact on the dose-response profile.

2.0 Effects in Pregnant Rats

Female rats, particularly pregnant rats, appear to be more sensitive than adult male rats to ChE inhibition caused by chlorpyrifos exposure (Moser et al. 1998 Hoberman 1998a, b, Mattsson et al. 1998, Lassiter et al. 1998, Zheng et al. 2000, Mendrala and Brzak 1998). Table 1 presents a comparison for both acute and repeat exposures. In mice, Weitman et al. (1983) found that PON1 activity towards the OP parathion was 50 nmol/min/ml in non-pregnant females, but it decreased as low as 14 nmol/min/ml during gestation (Weitman et al. 1983). Moser and Padilla (1998) found that inhibition of ChE in brain tissues had a sooner onset, a later peak effect, and a slower recovery in adult (approximately PND 70) females administered a single oral gavage dose of 80 mg/kg chlorpyrifos, compared to males.

Table 1. Comparison of Adult Pregnant Female and Male Cholinesterase Inhibition

Endpoint	Response	Comments
Acute ChEI - male and female rats (Mendrala and Brzak 1998; Lassiter et al. 1998a; Moser et al. 1998; Zheng et. al. 2000)	Male rats: slight (about 15%) brain ChEI at 10 mg/kg (2 studies); Male rats: 40% brain ChEI at 20 mg/kg; Female rats: 70% brain ChEI at 20 mg/kg; Female pregnant rats: 50% brain ChEI at 10 mg/kg	Pregnant female rats about 2-fold more sensitive than male rats to brain ChEI
Repeated Dose ChEI - male and female rats (Hoberman et al. 1998 a, b, MRID 44556901; Mattsson et al. 1998, MRID 44648101; Maurissen et al. 2000; Zheng et al. 2000)	Male rats, 14 days: $BMD_{10}/BMDL_{10}$: RBC ChEI: 0.2/0.095 mg/kg/day brain ChEI: 0.83/0.4 mg/kg/day Female pregnant rats GD6-20; 15 days (DNT): $BMD_{10}/BMDL_{10}$: RBC ChEI: 0.06/0.03 mg/kg/day brain ChEI: 0.65/0.55 mg/kg/day	Pregnant female rats more sensitive than male rats for RBC ChEI: RBC ChEI: 3.2-3.3-fold Brain ChEI: 1.2-fold (no sensitivity using $BMDL_{10}$)

DNT= developmental neurotoxicity study

3.0. Time Course Studies for Cholinesterase Inhibition

The Agency has examined time course information when evaluating AChE data, particularly the time of peak inhibition and how it is affected by age, duration and method of exposure, and other factors.

Table 2 shows some key studies with time to peak effect data for rat plasma, RBC, and/or brain ChE inhibition at various ages. Laboratories vary on the number of measurements and times following exposure when ChE inhibition is measured. In the available studies, the time to peak inhibition following exposure to chlorpyrifos varies from 2 to 24 hours, but it is typically between 3 and 6.5 hours. One study notes that the time for peak ChE inhibition in the brain of PND 1 pups following exposure to chlorpyrifos oxon was 1 hour; since this was the only available study on the oxon, it was not included in the table. The time to peak effect may vary with age, exposure regime, and other factors; therefore, time of measurement should be considered during comparison of data among different laboratories.

Table 2. Comparison of Time to Peak Effect for Chlorpyrifos in Rats

Study	Age	Sex	Dose (mg/kg)	Time of Peak ChE Inhibition		
				Plasma	RBC	Brain
Adults						
Mendrala and Brzak 1998	Adult	M	0.5	3 hr	NT	Not inhibited
Mendrala and Brzak 1998	Adult	M	1, 5, 10, 50, 100	6 hr	NT	10: 10 Hr (50/100: 12 hr)
Moser et al. 1998	Adult	M/F	20	NT	6.5 hr	6.5 hr
Moser and Padilla 1998	Adult	N/A	80	NT	3.5 hr	3.5 hr (males) 24 hr (females)
Pregnant Dams and Fetuses						
Ashry et al. 2002	Dam (at GD 18)	F	50	2-4 hr	NT	2 hr
Lassiter et al. 1998a	Dam (at GD 18)	F	7 (GD 14-18)	NT*	NT*	5 hr
Abu-Qare et al. 2001	Dam (at GD 18)	F	30 (dermal)	24 hr	NT	24 hr
Ashry et al. 2002	GD 18 (fetus)	N/A	50	4 hr	NT	4 hr
Lassiter et al. 1998a	GD 18 (fetus)	N/A	7 (GD 14-18)	NT	NT	5 hr
Abu-Qare et al. 2001	GD 18 (fetus)	N/A	30 (dermal)	NC	NT	24 hr
Postnatal Exposure to Pups						
Betancourt and Carr,2004	PND 1	M & F	1.5	NT	NT	12 hr
			3	NT	NT	4 hr
Timchalk et al. 2006	PND 5	N/A	1	3 hr	3 hr	3 hr
			10	6 hr	6 hr	
Timchalk et al. 2006	PND 12	N/A	1	24 hr	6 hr	6 hr
			10	6-24 hr		
	PND 17	N/A	1	24 hr	3-24 hr	24 hr
			10	6 hr	6-24 hr	
Moser and Padilla 1998	PND 17	M & F	15	NT	6.5 hr	6.5 hr
Moser et al. 1998		M & F	20			
	PND 27	M	20		6.5 hr	6.5 hr
		F	20		3.5 hr	3.5 hr
Dam et al. 2000	PND 1	N/A	1	2 hr	2 hr	2 hr
	PND 11	N/A	5	4 hr	4 hr	4 hr

N/A not applicable (sex not determined); NT= Not tested; NT* =whole blood assessed (2-10 hr peak); NC=No change

3.1. Adult rat

The registrant conducted a concentration-time course study of chlorpyrifos and chlorpyrifos-oxon in blood (Mendrela and Brzak 1998, MRID 44648102). Plasma ChE activity decreased in a time- and dose-dependent manner. The plasma ChE activities of rats treated with 0.5, 1, 5 or 10 mg/kg were maximally decreased 3-6 hours after treatment, with both the decrease and recovery of activity being dose-dependent. Plasma ChE activity was not significantly inhibited in the 0.5 mg/kg group. In the 1 mg/kg dose group, plasma ChE activity was significantly inhibited approximately 28% and 40% relative to controls at 3 and 6 hours post exposure, respectively. At 12 hours post-exposure (last time point measured), plasma ChE activity was still significantly inhibited about 15%. The decrease in plasma activity of rats treated with 50 or 100 mg/kg began within 10 minutes of treatment. At 12 hours after treatment, both groups were still significantly inhibited about 89% and had not shown signs of recovery.

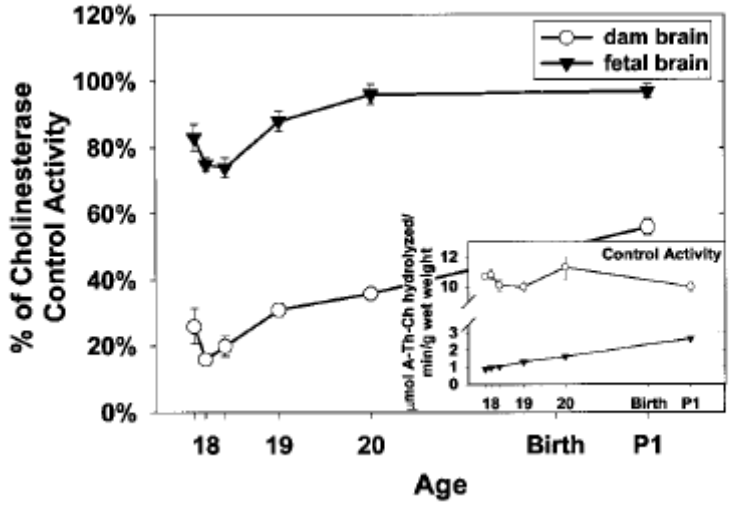
Brain AChE activity was not affected as dramatically by chlorpyrifos treatment as plasma activity with only the 10, 50, and 100 mg/kg dose groups showing significant effects. The brain AChE activity of rats treated with 10 mg/kg chlorpyrifos began to decline within three hours of treatment and was significantly decreased by six hours after treatment. The brain AChE activity in the 50 or 100 mg/kg dose groups decreased significantly within one hour of treatments; and by 12 hours (last time point measured), it was approximately 30% and 20%, respectively, of control. In none of the affected groups did brain ChE show signs of recovery.

3.2. Pregnant Dams and Fetuses

Ashry et al. (2002) gavaged (corn oil) dams with 50 mg/kg chlorpyrifos on GD 18 and then evaluated fetuses 1, 2, 4, 12 and 24 hours postdosing. For the fetus, the peak time of inhibition was 4 hours postdosing (87% for brain and 98% for plasma), and for the dams, it was between 2 and 4 hours (94% for brain and 95% for plasma). Although the times of peak effect were similar between the dams and fetuses, the levels of inhibition were high in both, resulting from exposure to a relatively high dose.

The Ashry study can be compared to the Abu-Qare et al. (2001) study, which has a similar timing and dose of exposure (single exposure to 30 mg/kg on GD 18). The major difference is that Abu-Qare et al. administered chlorpyrifos via the dermal route. Differences in absorption may have led to the delayed peak time for inhibition (24 hours), compared to the earlier peak following oral exposure. Abu-Qare et al. dermally exposed pregnant dams to a single 30 mg/kg chlorpyrifos dose on either GD 16, 17 or 18. Brain ChE was significantly inhibited at 27-33% in the fetus compared to 47-52% in the dams 48 and 24 hours after dosing on GD 16 and 17, respectively. The peak time for brain ChE inhibition was at 24 hours post dosing for both the fetus (33% decreased) and dam (52% decrease) treated on GD17, but was highest at 12 hours (compared to 2 and 4 hours) following exposure on GD18.

Lassiter et al. (1998a) conducted a study to compare the degree and define the time course of ChE inhibition in the dam, placenta, and fetus following repeated exposure late in gestation to chlorpyrifos. Chlorpyrifos was administered to Long Evans rats by gavage in corn oil at doses of 0 or 7 mg/kg/day on gestation days 14-18; animals were killed at 2, 5, 10, 24, 48, and 120 hours after the last dose. Recall that Hunter et al. (1999) found that the peak concentration of TCP in the fetal brain was twice that of the maternal brain when dams were exposed to 7 mg/kg/day on GD 14-18 (see Issue Paper & Appendix A), even though the half-lives for TCP in the maternal and fetal tissues were similar. In the Lassiter study, peak maternal blood and brain ChE inhibition occurred 5 hours after the last dose, and by 120 hours, ChE activity had recovered to 30-45% inhibition. Fetal brain ChE inhibition was inhibited less, with a maximum of 25% at 5 hours after last dose, and recovered to control levels by 48 hours. Comparison of the Lassiter and Ashry studies indicates that the time of peak brain ChE inhibition in the fetus is the same (5 hours) following repeated or acute oral exposure. In contrast, peak brain ChE inhibition in the dam is later following repeat exposure (5 hours), compared to a single dose in late gestation (2 hours).Figure from Lassiter, *et al.*, 1998.



3.3. Post-natal Exposure to Pups

In PND1 rats, the time of peak forebrain ChE inhibition following a single gavage dose was 12 hours at 1.5 mg/kg (58% decrease) and 4 hours at 3 mg/kg (82% decrease), indicating a shorter peak time with higher administered dose (Betancourt and Carr 2004). The peak time of ChE inhibition for chlorpyrifos oxon is less than for chlorpyrifos in PND1 pups, at about 1 hour postdosing, versus 4-12 hours for chlorpryrifos.

Timchalk et al. (2006) published an age-dependent pharmacokinetic and pharmacodynamic study for preweanling rats following rat exposure to chlorpyrifos. In the study, PND5, PND12 and PND 17 rats were given a single dose of 1 or 10 mg/kg chlorpyrifos via gavage (in corn oil), and plasma, RBC and brain ChE were measured at 3, 6 and 24 hours post dosing. Maximum inhibition was noted at 3-6 hours for PND5 rats at both doses. In PND 12 rats, peak inhibition time was about 6 hours for RBC and brain ChE activity and between 6 and 24 hours for plasma ChE. In PND 17 rats, the maximal brain ChE inhibition was 24 hours for both doses, but for plasma ChE activity was 24 hours at 1 mg/kg, and 6 hours at 10 mg/kg. RBC ChE showed maximal inhibition between 3-24 hours for both doses.

Moser and Padilla (1998) compared the effects of acute oral chlorpyrifos exposure in adult (70 days of age) and young (postnatal day 17) rats. They verified the findings of Pope, *et al.* (1991) that neonatal rats (10-27 days of age) were between 5-7 times more sensitive than adults to acute doses of chlorpyrifos at the maximum tolerated dose, with greater sensitivity identified in the youngest neonates. The time-course of the effects of an acute dose of chlorpyrifos was evaluated for adults and PND day 17 pups. Assessments included behavioral evaluations (functional observational battery and motor activity), ChE activity measurements, and muscarinic receptor assays. Doses were administered by gavage at levels that were selected to produce similar effects in young and adult rats; adults received 80 mg/kg and pups received 15 mg/kg. Following testing, tissues were taken at 1, 2, 3.5, 6.5, 24, 72, 168, or 336 hours post treatment. In adult rats, behavioral changes and brain and blood ChE inhibition followed the same temporal pattern. Peak effect occurred in male rats about 3.5 hours postdose. The onset of changes was more rapid in females, but the time-course was more protracted and recovery was slower. In pups, maximal behavioral effects, as well as ChE inhibition occurred 6.5 hours after dosing, without gender differences. Partial to full recovery of behavioral changes was observed at 24 and 72 hours, similar to adults. Blood and brain ChE inhibition in young rats had nearly recovered by 1 week postdose, but adult brain ChE had not fully recovered at 2 weeks. Muscarinic receptor binding assays showed apparent down-regulation in some brain areas at 24 and 72 hours after chlorpyrifos treatment. The study authors concluded that: 1) young rats show similar behavioral changes as adults, although at a 5-fold lower dose; 2) the onset of maximal effects is somewhat delayed in the young rats, 3) ChE activity tends to recover more quickly in young rats, but; 4) the young rats appear to have more extensive muscarinic receptor down-regulation; and 5) young rats show no gender-related differences.

In another publication, Moser et al. (1998) evaluated the age and gender-related differences in the sensitivity to chlorpyrifos in the rat. PND17, PND27 and adult (70 day) rats were given an acute oral dose of chlorpyrifos via gavage (in corn oil), and brain and blood ChE activity was measured 3.5 and 6.5 hours post-dosing to determine the differences in sensitivity to ChE inhibition. At 20 mg/kg, brain and blood ChE inhibition was slightly greater or the same at 6.5 hours when compared to 3.5 hr after dosing, with the exception of PND 27 females, which had slightly less inhibition at 6.5 hr.

In a study by (Pope, et al.1991), time course of ChE inhibition and recovery in whole brain was compared in neonatal (PND 7) and adult rats after treatment with maximal tolerated doses of chlorpyrifos (s. c. in peanut oil). Neonatal rats were more sensitive than adults with respect to lethality. Maximal brain ChE inhibition was similar in both age groups, but the ChE activity recovered faster in neonates.

3.4. Human Data

There are three single dose human studies that conducted time course cholinesterase measurements to determine the time to peak effect of cholinesterase inhibition (Nolan et al. 1982 Kisicki et al. 1999; Griffin et al. 1999). The time to peak effect for individuals in these studies is shown on Table 3, and indicates some variation in the human response to chlorpyrifos. More information on the deliberate dosing human studies can be found in Appendix G.

Table 3. Comparison of Time to Peak Effect for Chlorpyrifos Exposure in Humans

Study	Age/sex/dosing regimen	Dose (mg/kg)	Time of Peak Cholinesterase Inhibition	
			Plasma	RBC
Nolan et al. 1982	adult/male /single oral or dermal (n=6 oral/n=5 dermal)	0.5 (oral)	6 hr (1/6) (88%↓) 12 hr (3/6) (83-89%↓) 24 hr(2/6) (71-84%↓)	2 hr (1/6) (37%↓) 12 hr (1/6) (18%↓) 4 day (4/6) (14-53%↓)
		5 (dermal)	2-3 days (2/5) (21-35%↓)	None
Kisicki et al. 1999	adult/male and female (n=6/sex)/single oral	2	NT	12 hr (1/12) (28%↓)
Griffin et al. 1999	Adult/male and female (4 males/1 female)	0.1-0.014 (oral)	None	None
		0.31-0.39 (dermal)	None	None

An acute oral and dermal pharmacokinetic study (Nolan et al. 1982, MRID 00124144) dosed six men once with 0.5 mg/kg orally and four weeks later dosed five of these same men with 5 mg/kg dermally, and one man with 0.5 mg/kg dermally. Blood was collected 2, 6, 12 and 24 hours, and up to 30 days (oral) and up to 9 days (dermal) post dosing for plasma and RBC ChE measurements. No signs or symptoms were observed in any of the subjects, but the primary focus of this study was pharmacokinetics. Men orally exposed to 0.5 mg/kg chlorpyrifos exhibited peak plasma

ChE inhibition of 83%-89%, 6 to 24 hours post-exposure and peak RBC ChE inhibition of 14-53% on post-exposure day 4. One subject (E) displayed 37% RBC at 2 hours The return of plasma ChE activity to pre-dose levels required about 30 days. Men dermally exposed to 5 mg/kg chlorpyrifos exhibited peak plasma ChE inhibition of 21-35% on days 2- 3, and peak mean RBC ChE inhibition of 8-9% on day 4.

While RBC ChE inhibition was judged not to be significantly affected in the orally dosed group, mean values for the group reached a low point (a 27% decrease) on day 4. Individual values varied on this day between 14-53% of their pre-dose controls and a paired t-test comparing days 3 and 4 shows a statistically significant difference at a level of p=0.0115. The registrant stated that the inhibition noted on days 3 and 4 is an analytical artifact based on chlorpyrifos pharmacokinetics. If this is the case, it raises concerns about the quality and reliability of the study data. Again, HED notes that the relatively long recovery period of 30 days is unusual for plasma ChE, and is more characteristic of recovery for RBC acetyl ChE inhibition based on the 2 year dog data (McCollister et al. 1971, Kociba et al. 1985).

In a single-dose human oral toxicity study (Kisicki, et al. 1999/2000), 6 human subjects/sex/group were dosed orally with chlorpyrifos at dose levels of 0, 0.5, 1.0 in the first phase and 0 or 2.0 mg/kg in the second phase. Baseline measurements of red blood cell (RBC AChE) ChE activity were obtained for each subject and were used for comparison. RBC AChE was monitored at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours post dose. Plasma ChE was not assessed. No treatment-related effect was observed in males in RBC AChE activity at any dose level. In females, no treatment-related effect was observed in RBC AChE activity at the 0.5 and 1.0 mg/kg dose levels. At 2.0 mg/kg, one of the six females displayed RBC AChE inhibition (19%-28%) during the 8-48 hour time interval post dose. No assessments after 48 hours were made for this person. All other females at this dose level showed no inhibition.

In another human oral/dermal dosing toxicity study (Griffin et al. 1999), adults were exposed to a single-dose of analytical grade chlorpyrifos. Five subjects (4 males and 1 female) were dosed orally with 1 mg chlorpyrifos applied to a sugar cube. Four weeks after the oral dose, 28.59 mg of chlorpyrifos was administered to the skin of the same subjects by spreading 100 µL of a commercial preparation of chlorpyrifos diluted in water, onto an area of 78 cm2 of the inner forearm, which was then covered with a raised impermeable plastic container for 8 hours. Blood samples were collected over 24 hours. Plasma and erythrocyte (RBC) ChE activities were determined for each blood sample. Blood plasma and erythrocyte cholinesterase activity was never less that 90% of the pre-exposure values for either dosing regime.

4.0. Prenatal Exposure

In general, the available prenatal exposure data suggest that the dams have greater ChE inhibition than the fetus when measured at the same time point following *in utero* exposure to either a single or repeated dose. Refer to Appendix A for discussion of the age-related differences in ChE enzyme generation, in the rate of chlorpyrifos detoxification, as well as the levels of the terminal metabolites measured in the fetal and maternal tissues. A summary of some of the key studies are presented in Tables 4 and

5 and are discussed in greater detail below, beginning with single-dose studies and ending with repeated-dose studies submitted for registration and found in the open literature. The majority of the studies exposed dams to chlorpyrifos via oral gavage, but in some, chlorpyrifos was administered by subcutaneous injection.

4.1. Acute

Lassiter et al. (1998a) examined ChE inhibition in fetuses exposed to a single dose of 7 or 10 mg/kg chlorpyrifos on GD 18. As discussed in section 3.B, the level of inhibition measured in the fetuses was not different than the dams at 5 hours post-dosing for both dose groups. In the Lassiter study, both fetal and maternal brain ChE was inhibited about 40% at 5 hours following exposure to 10 mg/kg chlorpyrifos. At a lower dose of 7 mg/kg, there was no statistically significant ChE inhibition in either the fetal or dam brain.

Ashry et al. (2002) exposed dams to a much higher dose of chlorpyrifos, 50 mg/kg, by oral gavage on GD 18. At this dose, significant inhibition (65-67%) was already noted at 1 hr, the first time ChE was measured in both fetuses and dams. Maximal inhibition of brain AChE (about 95%) was reached at 4 hr in fetal brain, but not until 12 hr in maternal brain; recovery in the fetus was more rapid, whereas inhibition in the dam lasted from 12-48 hr. Similar results were observed, but with slower recovery, in plasma.

Subcutaneously injected (s.c.) chlorpyrifos to dams during gestation produces more extensive neurological effects in the dam relative to the developing fetus following a single high dose of 200 mg/kg/day on GD 12 (Chanda et al. 1995). However, as discussed below, there may appear to be less ChE inhibition at a given time point following exposure in the fetus, compared to adults, because the enzyme has a quicker recovery time due to its rapid rate of synthesis. In the Chanda study, it is possible that the ChE measurements were not taken at the time of peak effect as several days of recovery were allowed before ChE inhibition was measured. Dams had 82-88% brain ChEI on GD 16 (4 days post dosing) and PND 3, compared to 42-44% fetal brain ChEI at the same time measurements.

Similar results are seen following acute dermal exposure to chlorpyrifos, in that the level of ChE inhibition in the fetus when measured at a given time was less than that of the dam. Abu-Qare et al. (2001) dermally exposed pregnant dams to a single 30 mg/kg chlorpyrifos dose on either GD 16, 17 or 18. Brain ChE was significantly inhibited at 27-33% in the fetus compared to 47-52% in the dams 48 and 24 hours after dosing on GD 16 and 17, respectively. The peak time for brain ChE inhibition was at 24 hours post dosing for both the fetus (33% decreased) and dam (52% decrease) treated on GD17, but was highest at 12 hours (compared to 2 and 4 hours) following exposure on GD18.

Table 4. ChE inhibition1 following acute prenatal exposure to chlorpyrifos in rats.

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Fetal inhibition[2]	Compartment[3]	Maternal inhibition[2]
Abu-Qare et al. 2001	dermal *(acetone)*	GD 16	48 hrs after exposure	30 mg/kg	27% *		47% *
		GD 17	24 hrs after exposure		33% *		52% *
			12 hrs after exposure		23% (NS)		33% *
			4 hrs after exposure		19% (NS)		27% *
			2 hrs after exposure		16% (NS)		15% (NS)
Chanda et al. 1995	s.c. injection *(peanut oil)*	GD 12	GD 16	200 mg/kg	42% *	brain ChE	88%
Ashry et al. 2002	oral gavage *(corn oil)*	GD 18	1 hr after exposure	50 mg/kg	65% *	Brain AChE	67% *
					78% *	plasma AChE	81% *
			2 hrs after exposure		82% *	Brain AChE	94% *
					97% *	plasma AChE	95% *
			4 hrs after exposure		87% *	Brain AChE	84% *
					98% *	plasma AChE	94% *
			12 hrs after exposure		84% *	brain AChE	94% *
					98% *	plasma AChE	91% *
			24 hrs after exposure		90% *	brain AChE	95% *
					97% *	plasma AChE	91% *
Lassiter et al. 1998a	oral gavage *(corn oil)*	GD 18	5 hrs after exposure	7 mg/kg	19% (NS)	brain ChE	32% (NS)
				10 mg/kg	37%[4] *	brain ChE	51%[4]*

[1] **For many of the studies, inhibition levels were inferred from graphs.**
[2] **Levels are % inhibition, compared to controls.**
[3] **Compartments are given as described by the study author(s).**
[4] **Maternal and fetal compartment ChE inhibition are not significantly different from one another**
AChE = acetylcholinesterase
ChE = cholinesterase, reported as either total (acetyl- and butyryl) or not specified
GD = gestational day
N/A = not available
NS = not statistically significant
RBC = red blood cell
s.c. = subcutaneous
* $p<0.05$

4.2. Repeated Dosing, Studies Submitted for Registration

The text described in 4.2 is also summarized in Attachment A of this document.

In registrant-submitted developmental toxicity studies, chlorpyrifos toxicity was evaluated in rats, mice and rabbits. Two rat and one rabbit developmental study did not evaluate ChE inhibition in the fetus, so a comparison with the maternal ChE inhibition is not possible. In a mouse developmental study, the dams had significant plasma and RBC ChE inhibition at 1 mg/kg/day following exposure on gestation day 6-15, while fetus did not exhibit ChE inhibition until 10 mg/kg/day (Deacon et al. 1979, MRID 00095268). All of the available registrant repeated dosing studies indicate that the fetus has less ChE inhibition than the dams following repeated *in utero* exposure.

In the rat developmental neurotoxicity study, and a companion study (Hoberman 1998a, b, MRID 44556901; Mattsson et al. 1998, MRID 44648101; Mattsson et al. 2000; Maurissen et al. 2000), dams were treated with 0, 0.3, 1, or 5 mg/kg/day chlorpyrifos from GD 6 through lactation day 11. Statistically significant plasma (43-52%) and RBC (39-41%) ChE inhibition were noted in dams exposed to 0.3 mg/kg/day from gestation days 6-20 (4 hours postdosing). Brain ChE activity was significantly decreased in the 1 mg/kg/day (↓18%) and 5 mg/kg/day (↓90%) dams as compared to concurrent controls. No significant plasma, RBC or brain ChE inhibition was observed in the fetus on gestation day (GD) 20 or in the pups at any time measurement. In the high dose group (5 mg/kg/day), however, there was significant ChE inhibition on GD20 in the fetus (85%↓ plasma and 92%↓ RBC, 82% ↓heart, and 60%↓ brain). Slightly less ChE inhibition was noted in the pups exposed from GD 6 to postnatal day (PND) 1, with 60% plasma, 85% RBC, and 35% brain ChE inhibition 2 hours after dosing of the dams at 5 mg/kg/day. EPA evaluated this study and believes the pup ChE measurements at 2 hr postdosing dams is not an ideal time. On page 39 of MRID 44648102, it states that "most of the exposure from milk would likely have occurred between 3 and 6 hours post-maternal dosing (based on peak blood levels Fig.7) with a further delay of a few hours due to time necessary to digest the milk (based on Byczkowski et al. 1994)." Therefore, it appears that the ChE measurements underestimate the levels of inhibition that would be expected if the pups were allowed to be exposed to the majority of the chlorpyrifos in the milk.

4.3. Repeated Dosing, Literature Studies

Table 5 summarizes studies that show ChE inhibition following repeated prenatal exposure to chlorpyrifos in rats. As described above in the time course section, Lassiter et al. (1998a) conducted a study to compare the degree and define the time course of ChE inhibition in the dam, placenta, and fetus following late gestational exposure to chlorpyrifos following dosing on gestation days 14-18. In another study by Lassiter et al. (1999), dams were gavaged on GD 14-18 with 3, 5 or 10 mg/kg/day chlorpyrifos and evaluated 5 hours post-dosing. Fetal ChE was inhibited less than dams for all doses in the brain, liver and blood. Brain ChE was significantly depressed at 14% in the fetus and 33% in the dams of the 3 mg/kg/day group. Similar results were noted by Hunter et

al. (1999) where doses of 3 and 7 mg/kg/day during GD 14-18 resulted in more brain ChE inhibition in dams (41% and 84-87%) than the fetus (3% and 25-32% at 5 hour post dosing with chlorpyrifos.

Richardson and Chambers (2003) dosed pregnant rats orally via gavage (in corn oil) with 0, 3, 5, or 7 mg/kg chlorpyrifos from gestation day 6 to 20. Pups were sacrificed on postnatal days 1, 3, 6, 9 and 12 for determination of brain, heart, lung and serum ChE activities. Cholinesterase inhibition in the dams was not measured. Cholinesterase activities were inhibited in a dose-related manner, with brain cholinesterase inhibition of about 26%, 32%, and 45% in the 3, 5, and 7 mg/kg/day groups, respectively, on PND 1. Inhibition of brain ChE persisted in all treatment groups until postnatal day 6, and in the 3 and 7 mg/kg dose groups until PND 9. Lung ChE was maximally inhibited on PND1 with inhibition of about 28%, 74% and 75% in the 3, 5 and 7 mg/kg/day groups, respectively. Serum ChE was inhibited to a similar degree in all dosage groups, about 30%, 35% and 32% on PND1.

Four repeated prenatal exposure studies can be compared to examine the effect of administration method on ChE inhibition. In Hunter et al. (1999), fetal brain ChE was inhibited 12% on GD 19, 24 hours following exposure to 7 mg/kg/day via oral gavage on GD 14-18. Lassiter et al. (1998) had a similar exposure regime and found fetal brain ChE to be inhibited 17% on GD 19, 24 hours following exposure to 7 mg/kg/day via oral gavage on GD 14-18. In comparison, Chanda and Pope (1996) measured 40% fetal brain ChE inhibition in GD 20 pups whose dams were exposed via s.c. injection to a slightly lower dose, 6.25 mg/kg/day, on GD 12-19. Although greater inhibition of fetal brain ChE activity was seen in the older pups in the study with longer exposure, the levels of maternal brain ChE inhibition were similar at these same time points: 68% in the Hunter study, 74% in the Lassiter study, and 75% in the Chanda and Pope study. Qiao et al. (2002) also exposed dams via s.c. injection, and found that 5 mg/kg/day on GD 17-20 resulted in 44-50% inhibition of fetal brain ChE in GD 21 pups. Greater levels of fetal inhibition were seen following s.c. injection, but this increased inhibition could also be due to the older age of the fetuses. Comparison of the maternal ChE data shows no difference in inhibition due to route of exposure.

Two studies were examined in which pregnant rats were exposed to repeated doses in the range of 20-25 mg/kg/day chlorpyrifos and ChE measured 24 hours after exposure. When dams were exposed to 25 mg/kg/day via s.c. injection on GD12-15, fetal brain ChE activity was inhibited 42% when measured on GD 16, 24 hours after exposure (Chanda and Pope 1996). Exposure to 25 mg/kg/day on GD 12-19 by s.c. injection caused 58% inhibition of fetal brain ChE when measured on GD 20, 24 hours after exposure (Chanda and Pope 1996). Oral exposure by the dams to 20 mg/kg/day chlorpyrifos on GD17-20, caused 74% and 82% inhibition of fetal brainstem and forebrain ChE activity, respectively, when measured on GD 21, 24 hours after the last dose (Qiao et al. 2002). For all of these exposure regimes, maternal brain ChE inhibition was greater than in the fetus. Comparison of the brain ChE inhibition levels on GDs 16, 20, and 21 suggests that the older fetus is more sensitive than the younger fetus, with the dam more sensitive than the fetus of either age. However, Lassiter et al. (1998a) reported that the amount of fetal brain ChE activity increased 4.3 times between GD14 and GD18. The authors speculate that, “it may be that the new

synthesis of uninhibited cholinesterase molecules may dilute the inhibited molecules". Thus, the difference in fetal brain ChE inhibition between GD16 and GD21 following exposure to similar doses for 4-7 days, with greater inhibition seen later in gestation compared to mid-gestation, may be due to a difference in the rate of recovery.

In both Mattsson et al. (2000) and Lassiter et al. (1999), dams were exposed to 5 mg/kg/day chlorpyrifos via oral gavage and examined 4-5 hours after the final dose. In the Lassiter study, animals were exposed from GD 14-18 and measured 5 hours after exposure, and in the Mattsson study, they were exposed from GD 6-20 and measured 4 hours after exposure. Brain ChE inhibition was 30% in GD 18 fetuses (Lassiter et al. 1999), and hindbrain and forebrain ChE inhibition was 56-60% in GD 20 fetuses (Mattsson et al. 2000). The higher degree of inhibition in the Mattsson et al. (2000) study may have resulted from the longer exposure duration, the difference in time of measurement, and/or the difference in enzyme synthesis rate between the younger and older fetus.

Table 5. ChE inhibition[1] following repeated prenatal exposure to chlorpyrifos in rats.

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Fetal inhibition[2]	Compartment[3]	Maternal inhibition[2]
Mattsson et al. 1998, 2000[5]	oral gavage *(corn oil)*	GD 6-20	4 hrs after exposure (GD 20)	0.3 mg/kg/day	0%	forebrain ChE	2% (NS)
					0%	hindbrain ChE	0%
					0%	RBC ChE	24% **
					0%	plasma ChE	35% **
					0%	heart ChE	0%
				1 mg/kg/day	8% (NS)	forebrain ChE	10%/7%[5] **
					0%	hindbrain ChE	12%/7% [5] (NS)
					5% (NS)	RBC ChE	87%/85% [5] **
					4% (NS)	plasma ChE	77%/60% [5] **
					0%	heart ChE	49%/50% [5] **
				5 mg/kg/day	60% **	forebrain ChE	89%/85% [5] **
					56% **	hindbrain ChE	80%/75%[5] **
					92% **	RBC ChE	99%/95% [5] *
					85% **	plasma ChE	94%/85% [5] **
					82%**	heart ChE	89%/80% [5] **
Hoberman et al., 1998 a, b, Maurissen et al. 2000	oral gavage *(corn oil)*	GD 6-20	4 to 5 hrs after exposure (GD 20)	0.3 mg/kg/day	N/A	plasma ChE	43.3%
					N/A	RBC ChE	41.3%
					N/A	brain ChE	0.3%
				1 mg/kg/day	N/A	plasma ChE	68.9%
					N/A	RBC ChE	84.4%
					N/A	brain ChE	17.9%
				5 mg/kg/day	N/A	plasma ChE	91.5%
					N/A	RBC ChE	99.9%
					N/A	brain ChE	89.8%
Richardson and Chambers 2003	oral gavage *(corn oil)*	GD 6-20	PND 1	3 mg/kg/day	26% *	brain ChE	N/A
					30% *	serum ChE	N/A
					28%*	lung ChE	N/A
				5 mg/kg/day	32% *	brain ChE	N/A
					35% *	serum ChE	N/A
					74% *	lung ChE	N/A
				7 mg/kg/day	45% *	brain ChE	N/A
					32% *	serum ChE	N/A
					75% *	lung ChE	N/A
Mattsson et al. 1998, 2000	oral gavage *(corn oil)*	GD 6-PND 1	2 hrs after exposure (PND 1)	0.3 mg/kg/day	0%	forebrain ChE	0%
					0%	hindbrain ChE	7% (NS)
					0%	RBC ChE	40% **
					0%	plasma ChE	55% **
					0%	heart ChE	10% (NS)
				1 mg/kg/day	5% (NS)	forebrain ChE	6% (NS)
					0%	hindbrain ChE	6% (NS)
					0%	RBC ChE	90% **
					5% (NS)	plasma ChE	80% **
					2% (NS)	heart ChE	40% **
				5 mg/kg/day	35% **	forebrain ChE	88% **
					35% **	hindbrain ChE	80% **
					85% **	RBC ChE	99% **
					60% **	plasma ChE	95% **
					65% **	heart ChE	85% **

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Fetal inhibition[2]	Compartment[3]	Maternal inhibition[2]
Chanda and Pope 1996	s.c. injection *(peanut oil)*	GD 12-15	24 hrs after exposure (GD 16)	25 mg/kg/day	42% *	brain ChE	82% **
Chanda and Pope 1996	s.c. injection *(peanut oil)*	GD 12-19	24 hrs after exposure (GD 20)	6.25 mg/kg/day	40% *	brain ChE	75% *
				12.5 mg/kg/day	50%*		85% *
				25 mg/kg/day	58% *		90% *
Lassiter et al. 1998a	oral gavage *(corn oil)*	GD 14-18	2 hrs after exposure (GD 18)	7 mg/kg/day	17% (p value N/A)	brain ChE	74% (p value N/A)
					38% (p value N/A)	liver ChE	85% (p value N/A)
			5 hrs after exposure (GD 18)		25% (p value N/A)	brain ChE	84% (p value N/A)
					46% (p value N/A)	liver ChE	84% (p value N/A)
			10 hrs after exposure (GD 18)		26% (p value N/A)	brain ChE	80% (p value N/A)
					46% (p value N/A)	liver ChE	82% (p value N/A)
			24 hrs after exposure (GD 19)		12% (p value N/A)	brain ChE	69% (p value N/A)
					15% (p value N/A)	liver ChE	58% (p value N/A)
Lassiter et al. 1999	oral gavage *(corn oil)*	GD 14-18	5 hrs after exposure (GD 18)	3 mg/kg/day	14%	brain ChE	33%
					26%	liver ChE	78%
					NA	blood ChE	88%
				5 mg/kg/day	30%	brain ChE	70%
					52%	liver ChE	80%
					NA	blood ChE	93%
				7 mg/kg/day	45%	brain ChE	86%
					67%	liver ChE	84%
					NA	blood ChE	94%
				10 mg/kg/day	47%	brain ChE	89%
					75%	liver ChE	89%
					NA	blood ChE	96%
Hunter et al. 1999	oral gavage *(corn oil)*	GD 14-18	5 hrs after exposure (GD 18)	3 mg/kg/day	3%	brain ChE	41%
					24%	liver ChE	84%
			2 hrs after exposure (GD 18)	7 mg/kg/day	16%	brain ChE	74%
					38%	liver ChE	85%
			5 hrs after exposure (GD 18)		25% & 32% [4]	brain ChE	84% & 87% [4]
					43% & 60% [4]	liver ChE	84% & 89% [4]
			10 hrs after exposure (GD 18)		26%	brain ChE	80%
					43%	liver ChE	82%
			24 hrs after exposure (GD 19)		12%	brain ChE	68%
					16%	liver ChE	58%
Qiao et al. 2000	s.c. injection *(DMSO)*	GD 17-20	GD 21	1 mg/kg/day	3% (NS)	brainstem ChE	N/A
					6% (NS)	forebrain ChE	N/A
				2 mg/kg/day	15% ***	brainstem ChE	N/A
					20% ***	forebrain ChE	N/A
				5 mg/kg/day	44%	brainstem ChE	N/A
					50%	forebrain ChE	N/A

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Fetal inhibition[2]	Compartment[3]	Maternal inhibition[2]
				10 mg/kg/day	65%	brainstem ChE	N/A
					75%	forebrain ChE	N/A
				20 mg/kg/day	74%	brainstem ChE	N/A
					82%	forebrain ChE	N/A
				40 mg/kg/day	78%	brainstem ChE	N/A
					84% (see graph A)	forebrain ChE	N/A

[1] For many of the studies, inhibition levels were inferred from graphs.
[2] Levels are % inhibition, compared to controls.
[3] Compartments are given as described by the study author(s).
[4] This study was broken into dose-response and time-course components, and each measured brain and liver ChE 5 hrs after exposure to 7 mg/kg/day.
[5] Two maternal inhibition values are presented. The first value represents EPA independently derived values from analysis of the raw data as presented in the EPA Data Evaluation Record (DER), and the second value is that published in the literature study.

ChE = cholinesterase, reported as either total (acetyl- and butyryl) or not specified
DMSO = dimethyl sulfoxide
GD = gestational day
N/A = not available
NS = not statistically significant
PND = postnatal day
RBC = red blood cell
s.c. = subcutaneous
* $p<0.05$
** $p<0.02$
*** $p<0.0001$

5.0. Postnatal Exposure

Several studies exist in which a single dose of chlorpyrifos was administered directly to the pup, via oral gavage, s.c. injection, or i.p. injection, at ages ranging from PND 1 to PND 33. The text summarized in 5.1 and 5.2 is tabulated in Table 6. Generally, it appears than younger pups are more susceptible to ChE inhibition than older pups.

Repeated exposure studies also exist where chlorpyrifos was administered via oral gavage or s.c. injection on two or more days, beginning as early as PND 1. These studies are summarized in 5.3 and 5.4 and tabulated below in Table 7. It may be important to consider the time of measurement, as well as the beginning time of exposure.

The Agency has conducted benchmark dose (BMD) analysis on several of the single dose studies for consideration as an acute point of departure. A summary of the BMD analysis is presented in Section 6 below.

5.1. Acute Oral

Pups were administered 1.5 or 3 mg/kg chlorpyrifos via gavage in corn oil on PND 1 (Betancourt and Carr 2004). The time of peak forebrain ChE inhibition in PND 1 rats following a single dose was 12 hours at 1.5 mg/kg (58% decrease) and 4 hours at 3 mg/kg (82% decrease), indicating a shorter peak time with higher administered dose.

Timchalk et al. (2006) published an age-dependent pharmacokinetic and pharmacodynamic response model for preweanling rats following rat exposure to chlorpyrifos. In the study, PND 5 , PND 12, and PND 17 rats were given a single dose of 1 or 10 mg/kg chlorpyrifos via gavage (in corn oil), and plasma, RBC, and brain ChE levels were measured at 3, 6, and 24 hours post dosing. At both dose levels, younger animals demonstrated a greater sensitivity to plasma, RBC, and brain ChE inhibition (sensitivity was PND5>PND12>PND17). Maximum inhibition was noted at 3-6 hours for PND 5 rats for all compartments. In older PND 17 rats, the time for maximal ChE inhibition was longer; peak brain and plasma ChE inhibition was measured at 24 hours, and peak RBC ChE inhibition was between 3-24 hours for both doses. At 1 mg/kg, maximal plasma ChE inhibition was 62%, 33.4%, and 21.9% for PND 5, 12 and 17 rats, respectively, while maximal RBC ChE inhibition was 45.7%, 27%, and 15%, respectively. Maximal brain ChE inhibition at 1 mg/kg was 22.1%, 5.2%, and 2.5%, for PND 5, 12, and 17 rats, respectively. Dr. Charles Timchalk provided the individual animal data from this study with anticipation that the Agency would perform benchmark dose (BMD) modeling on these data. Prior to the conduct of the BMD analysis, the Agency identified some inconsistencies in these data. For example, in the brain AChE data in PND5 animals at each timepoint, 3-4 animals exhibited approximately 4X more activity than the remaining animals in the group. If this is resolved, the Agency may, in the future, use the PND 5 data in a BMD analysis. BMD results for PND 12 and 17 are provided below.

Zheng et al. (2000) conducted a comparative ChE study between adult male rats and neonatal rats on postnatal day 7. Animals were acutely dosed with chlorpyrifos via gavage (in peanut oil) with 0, 0.15, 0.45, 0.75, 1.5, 4.5, 7.5, or 15 mg/kg, and ChE measurements were performed 4 hours post-dosing. ChE activity in neonates was inhibited similarly in plasma, RBC, and the frontal cortex (ED_{50}=1.5-2.9 mg/kg) while in adults, significant ChE inhibition was noted only in plasma and RBC. In the adult males, no significant ChE inhibition was noted at 0.75 mg/kg/day, while at 1.5 mg/kg, there was significant 23.7% plasma and 29.7% RBC ChE inhibition. The neonatal pups were more sensitive to ChE inhibition following a single oral dose, with approximately 14% RBC ChE inhibition at 0.45 mg/kg and 0.75 mg/kg, and statistically significant 31.8% RBC and 17% brain ChE inhibition at 1.5 mg/kg. Similar to the Timchalk et al. (2006) study, the Agency secured additional information from Dr. Carey Pope that was not published in the Zheng et al. (2000) paper. With this information, the Agency performed a preliminary BMD analysis on the pup data (both acute and repeated). These data are highly variable at the low end of the dose-response curve. Specifically, the results show 33% increase, 13.7% decrease, and 23% increase in brain ChE activity at the 0.15, 0.45, and 0.75 mg/kg doses, respectively. Because of this significant variability, the BMD results at the 10% response level typically used by OPP to derive points of departure (PoD) in risk assessment are highly variable and considered unreliable for brain ChE inhibition.

Moser et al. (1998) evaluated the age- and gender-related differences in the sensitivity to chlorpyrifos in the rat. PND 17, PND 27 and adult (70 day) rats were given an acute oral dose of chlorpyrifos via gavage (in corn oil), and brain and blood ChE activity was measured 3.5 and 6.5 hours post-dosing to determine the differences in

sensitivity to ChE inhibition. (In addition, the study evaluated differences in behavioral changes.) PND 17 rats were given 5 or 20 mg/kg chlorpyrifos, PND27 rats were given 20 or 50 mg/kg chlorpyrifos, and adult rats were given 20 or 80 mg/kg chlorpyrifos. Comparisons of the 20 mg/kg dose across age groups showed generally less ChE inhibition and fewer behavioral effects with increasing age, with the exception that adult females were similar to the PND 27 rats. The degree of ChE inhibition in the brain more closely paralleled the blood inhibition in the younger rats, compared to the adults. Preweanling rats had considerably less carboxylesterase (CarbE) and A-esterase activity, and adult females had less liver CarbE activity than males. These differences in detoxifying enzymes correlate with the age-related differences in behavioral and biochemical effects, as well as the gender differences seen in adult rats, and the authors conclude may be a major influence on the differential sensitivity of chlorpyrifos.

In a more recent publication, Moser et al. (2006), 17 day old male rats were exposed to a single dose via gavage (in corn oil) of 0, 0.5, 2, 5, 10, or 20 mg/kg chlorpyrifos. Blood and brain ChE activity was measured between 4.25 and 4.5 hours post-dosing. In this study, blood ChE inhibition was approximately 10%, 40%, 80%, 89% and 96.5%, while brain ChE inhibition was 0, 10%, 58%, 70% and 80% for the 0.5, 2, 5, 10 and 20 mg/kg dose groups, respectively. Dr. Ginger Moser has provided the individual animal data for BMD analysis, and the results are shown in Section 6.

5.2. Acute, Subcutaneous Injection

Dam et al. (2000) subcutaneously injected 1 or 5 mg/kg chlorpyrifos to PND 1 or PND 11 male and female rats, respectively, and measured brain ChE activity at 2 and 4 hours post dosing. In the PND1 pups, brain ChE inhibition was greater at 2 hours post dosing (60-80% for males and 10-35% for females) than at 4 hours post dosing (35-50% for males and 0-25% for females), and it was also greater in males than females. In the PND 11 pups, the degree of ChE inhibition was much lower than observed in PND 1 rats, even though they were exposed to a higher dose, there were no apparent sex differences, and ChE inhibition generally increased between 2 and 4 hours.

Jett et al. (2001) dosed PND 7 rats with 0.3 and 7 mg/kg chlorpyrifos subcutaneously in peanut oil and reported no significant ChE in the cerebellum, cortex and hippocampus 3 and 24 hours post-dosing. Blood AChE was not measured. Since a dose of 7 mg/kg chlorpyrifos was given orally to 17 day old rats would be expected to result in about 60% brain ChE inhibition (Moser et al., 2006), there is an open question as to whether or not the method of administration (subcutaneous in peanut oil) resulted in inhibition that was not detected during the times for assessment. The results of Jett et al. (2001) are discussed in more detail in the Issue Paper and Appendix C.

Table 6. ChE inhibition[1] following acute postnatal exposure[2] to chlorpyrifos in rats.

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
Dam et al. (2000)	s.c. injection *(DMSO)*	PND 1	2 hrs after exposure	1 mg/kg	70% (M); 25% (F)	brainstem ChE
					80% (M); 10% (F)	cerebellum ChE
					60% (M); 35% (F)	forebrain ChE
			4 hrs after exposure	1 mg/kg	35% (M); 25 % (F)	brainstem ChE
					40 % (M); 0 % (F)	cerebellum ChE
					50 % (M); 25 % (F)	forebrain ChE
Atterberry et al. (1997)	i.p. injection *(corn oil)*	PND 1	2 hrs after exposure	80 mg/kg	>90% *	cerebral cortex & medulla oblongata/pons AChE
Betancourt and Carr (2004)	oral gavage *(corn oil)*	PND 1	4 hrs after exposure	1.5 mg/kg	25%	forebrain ChE
			8 hrs after exposure		28%	
			10 hrs after exposure		40%	
			12 hrs after exposure		58%	
			24 hrs after exposure		49%	
			4 hrs after exposure	3 mg/kg	82%	forebrain ChE
			8 hrs after exposure		81%	
			10 hrs after exposure		80%	
			12 hrs after exposure		81%	
			24 hrs after exposure		54%	
Atterberry et al. (1997)	i.p. injection *(corn oil)*	PND 3	2 hrs after exposure	80 mg/kg	>90% *	cerebral cortex & medulla oblongata/pons AChE
Timchalk et al. (2006)	oral gavage *(corn oil)*	PND 5	3 hrs after exposure	1 mg/kg	22.1%	brain ChE
			3 hrs after exposure		45.7%	RBC ChE
			3 hrs after exposure		62.1%	plasma ChE
			3 hrs after exposure	10 mg/kg	83.6%	brain ChE
			6 hrs after exposure		83.5%	RBC ChE

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
			6 hrs after exposure		98.4%	plasma ChE
Jett et al. (2001)	s.c. injection *(peanut oil)*	PND 7	3 & 24 hrs after exposure	0.3 mg/kg	Ns	cerebellum ChE
					Ns	cortex ChE
					Ns	Hippocampus ChE
				7 mg/kg	Ns	cerebellum ChE
					Ns	cortex ChE
					Ns	Hippocampus ChE
Zheng et al. (2000)	oral gavage *(peanut oil)*	PND 7	4 hrs after exposure	0.15 mg/kg	33% increase	frontal cortex ChE
					3% increase	RBC ChE
					11%	plasma ChE
				0.45 mg/kg	13.7	frontal cortex ChE
					15%	RBC ChE
					27%	plasma ChE
				0.75 mg/kg	23% increase	frontal cortex ChE
					15%	RBC ChE
					38%	plasma ChE
				1.5 mg/kg	17%	frontal cortex ChE
					32%	RBC ChE
					51%	plasma ChE
				4.5 mg/kg	77%	frontal cortex ChE
					84%	RBC ChE
					77%	plasma ChE
				7.5 mg/kg	77%	frontal cortex ChE
					88%	RBC ChE
					78%	plasma ChE
				15 mg/kg	93%	frontal cortex ChE
					94%	RBC ChE
					76%	plasma ChE
Won et al. (2001)	oral gavage *(peanut oil)*	PND 7	4 hrs after exposure	7.5 mg/kg	48% *	frontal cortex ChE
				15 mg/kg	N/A	
Pope and Chakraborti (1992)	s.c. injection *(peanut oil)*	PND 7	24 hrs after exposure	15 mg/kg	40% (p value N/A)	brain ChE
				19.9 mg/kg	50% (p value N/A)	plasma ChE
Pope et al. (1991)	s.c. injection *(peanut oil)*	PND 7	4 hrs after exposure	45 mg/kg	40% (p value N/A) (can't verify)	plasma ChE
			24 hrs after exposure		78% (p value N/A)	brain ChE
					93% (p value N/A)	RBC ChE
					94% (p value N/A)	plasma ChE
Dam et al. (2000)	s.c. injection *(DMSO)*	PND 11	4 hrs after exposure	5 mg/kg	15% (M); 30% (F)	brainstem ChE
					35% (M); 25% (F)	cerebellum ChE
					20% (M); 20% (F)	forebrain ChE
Timchalk et	oral gavage	PND 12	6 hrs after exposure	1 mg/kg	5.2%	brain ChE

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
al. (2006)	*(corn oil)*		6 hrs after exposure		27.0%	RBC ChE
			24 hrs after exposure		33.4%	plasma ChE
			6 hrs after exposure		80.4%	brain ChE
			6 hrs after exposure	10 mg/kg	80.9%	RBC ChE
			6 & 24 hrs after exposure		87%	plasma ChE
Atterberry et al. (1997)	i.p. injection *(corn oil)*	PND 12	2 hrs after exposure	80 mg/kg	>90% *	cerebral cortex & medulla oblongata/pons AChE
Timchalk et al. (2006)	oral gavage *(corn oil)*	PND 17	24 hrs after exposure		2.1%	brain ChE
			3 & 24 hrs after exposure	1 mg/kg	15%	RBC ChE
			24 hrs after exposure		21.9%	plasma ChE
			24 hrs after exposure		58.9%	brain ChE
			6 & 24 hrs after exposure	10 mg/kg	62%	RBC ChE
			6 hrs after exposure		75.7%	plasma ChE
Moser et al. 2006	oral gavage *(corn oil)*	PND17	4.5 hours postdosing	0.5	0%	Brain ChE
				2	10%	
				5	58%	
				10	70%	
				20	80%	
				0.5	10%	Blood ChE
				2	40%	
				5	80%	
				10	89%	
				20	96.5%	
Moser et al. (1998)	oral gavage *(corn oil)*	PND 17	3.5 hrs after exposure	5 mg/kg	73% (M); 70% (F) (p value N/A)	blood ChE
			6.5 hrs after exposure		62% (M); 60% F) (p value N/A)	brain ChE
					80% (M); 77% (F) (p value N/A)	blood ChE
			3.5 hrs after exposure	20 mg/kg	87% (M); 87% (F)	Brain ChE
			6.5 hrs after exposure		89% (M); 91% (F)	
Chanda et al. (2002)	oral gavage *(corn oil)*	PND 17	3.5 hrs after exposure	15 mg/kg	77% (p value N/A)	plasma ChE
			6.5 hrs after exposure		85% (p value N/A)	brain ChE

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
					80% (p value N/A)	liver ChE
Moser and Padilla (1998)	oral gavage *(corn oil)*	PND 17	3.5 hrs after exposure	15 mg/kg	86% (M); 82% (F) (p value N/A)	RBC ChE
			6.5 hrs after exposure		87% (p value N/A)	brain ChE
			24 hrs after exposure		91% (M); 90% (F) (p value N/A)	RBC ChE
					80%*	diaphragm ChE
					80% *	heart ChE
Won et al. (2001)	oral gavage *(peanut oil)*	PND 21	4 hrs after exposure	23.5 mg/kg	65% *	frontal cortex ChE
Moser et al. (1998)	oral gavage *(corn oil)*	PND 27	3.5 hrs after exposure	20 mg/kg	89% (M); 90% (F) (p value N/A)	blood ChE
			6.5 hrs after exposure		70% (M); 69% (F) (p value N/A)	brain ChE
					89% (M); 88% (F) (p value N/A)	blood ChE
			3.5 hrs after exposure	50 mg/kg	86% (M); 80% (F)	Brain ChE
			6.5 hrs after exposure		80% (M); 88% (F)	
Atterberry et al. (1997)	i.p. injection *(corn oil)*	PND 33	2 hrs after exposure	80 mg/kg	50% *	cerebral cortex & medulla oblongata/pons AChE

[1] For many of the studies, inhibition levels were inferred from graphs.
[2] Data only provided for studies in which pups were directly dosed; data from lactational transfer studies were not included.
[3] Levels are % inhibition, compared to controls.
[4] Compartments are given as described by the study author(s).

AChE = acetylcholinesterase
ChE = cholinesterase, reported as either total (acetyl- and butyryl) or not specified
DMSO = dimethyl sulfoxide
F = female
i.p. = intraperitoneal
M = male
N/A = not available
Ns = not significant
PND = postnatal day
RBC = red blood cell
s.c. = subcutaneous
* $p<0.05$

5.3. Repeated Oral

The Agency has conducted benchmark dose (BMD) analysis on several of the repeated studies for consideration as a chronic point of departure. A summary of the BMD analysis is presented in Section 6 below.

In the open literature, ChE inhibition has been observed at doses as low as 1 mg/kg/day following repeated exposure to young pups. Guo-Ross et al. (2007) exposed pups to chlorpyrifos on PNDs 1-4 via oral gavage. When measured on PND 4, 4 hours after chlorpyrifos exposure, brain ChE was inhibited 25% at 1 mg/kg/day and 45% at 1.5 mg/kg/day. Betancourt and Carr (2004) also exposed young pups, on PNDs 1-3 via oral gavage, to 1.5 mg/kg/day. At this dose, they found less inhibition than Guo-Ross, 27% in the forebrain, but their measurement was taken at 24 hours after the last dose, rather than at 4 hours, and likely missed peak inhibition. Betancourt and Carr (2004) found greater brain ChE inhibition with 3 mg/kg/day (45%), but this measurement was again taken 24 hours after the last dose. In older rats exposed from PND1-11, there was no forebrain ChE inhibition at 1.5 mg/kg, and 20% ChE inhibition at 3 mg/kg, 24 hours post dosing (Betancourt and Carr, 2004).

Richardson and Chambers (2005) examined ChE inhibition in several ages of developing rats following repeated oral exposure to chlorpyrifos. Rats were gavaged (in corn oil) daily from postnatal day (PND) 1-12 with 1.5 mg/kg, and increasing gradually to 3 mg/kg and then to 6 mg/kg. Brain ChE activity was significantly inhibited on PND 6, 12, 22 and 30. Following daily doses of 1.5 mg/kg daily, PND 6, PND 12, PND 22, and PND 30 rats had 49%, 43%, 36%, and 18% brain ChE inhibition, respectively. ChE measurements were 6 hours, 12 hours, 24 hours and 9 days post dosing for PND 6, 12, 22 and 30 rats, respectively. On PND 22 and 30, 94% or greater of the inhibited ChE could not be reactivated by the oxime TMB-4 in both treatment groups, indicating aging of the phosphorylated ChE. The authors conclude that the long-term reduction in brain ChE activity that was observed following repeated postnatal exposure to chlorpyrifos is attributable to permanent inactivation or “aging” of the enzyme.

In a 14 day, repeated comparative cholinesterase study between adult male rats and neonatal rats (PND 7- 20) animals were dosed with chlorpyrifos via gavage (in peanut oil) with 0, 0.15, 0.45, 0.75, 1.5, 4.5, 7.5 or 15 mg/kg, and ChE measurements were performed 4 hours post dosing (Zheng et al. 2000). ChE activity in neonates was inhibited similarly in plasma, RBC and the frontal cortex, while in adults, significant ChE inhibition was noted only in plasma and RBC. Neonates were not substantially more sensitive to ChE inhibition following repeated exposure, with the possible exception of brain ChE inhibition. For example, brain ChE was inhibited 41.9% in neonates and 23% in adult males at 1.5 mg/kg/day. However, the Agency notes that the brain ChE activity data for this study are highly variable, which reduces the confidence in these results. The study authors suggest that the relative “resistance” of neonates to repeated exposures is a more robust recovery of ChE activity following each exposure. As noted above, the Agency attempted a BMD analysis of this study, but due to substantial variability at doses lower than 1.5 mg/kg/day, the BMDs at the 10% response level were considered unreliable for brain ChE inhibition.

5.4. Repeated Subcutaneous Injection

Song et al. (1997) exposed young rat pups from PND 1-4 to 1 mg/kg/day chlorpyrifos via s.c. injection. Brainstem ChE was inhibited 24% on PND 5, 24 hours after the last dose. This data correlates with the 27% inhibition of forebrain ChE noted on PND 4 following oral exposure to 1.5 mg/kg/day on PNDs 1-3 (Betancourt and Carr, 2004), and the 25% inhibition of brain ChE noted 4 hours after oral exposure to 1 mg/kg/day on PNDs 1-3 (Guo-Ross, 2007). These data indicate that similar levels of brain ChE inhibition are reached in young pups following repeat exposure, whether chlorpyrifos is administered via oral gavage or s.c. injection.

Jett et al. (2001) dosed rats on postnatal days 7, 11, and 15 with 0.3 and 7 mg/kg chlorpyrifos subcutaneously in peanut oil and reported no significant ChE in the cerebellum, cortex and hippocampus on PND 16. In contrast, Liu et al. (1999) administered 5 mg/kg/day chlorpyrifos via s.c. injection to similarly aged pups, from PND 7-13, and reported 64% and 74% inhibition of cortex and striatum ChE, respectively, when measured on PND 14, 24 hours after the last dose. In the Jett study, rats dosed on PND22 and 26 also did not exhibit ChE inhibition in the cerebellum, cortex and hippocampus 2 days (on PND 28) after dosing. When Liu et al. (1999) extended dosing to 5 mg/kg/day on PNDs 7-20, they measured 60% and 68% inhibition of cortex and striatum ChE, respectively, on PND 21. The differences between these studies is not likely due to the “recovery” of the pups between doses in the Jett study, as a similar dose of 10 mg/kg/day, by oral gavage, still resulted in 59% inhibition of brain ChE activity on PND 17 (Timchalk, 2006).

Table 7. ChE inhibition[1] following repeated postnatal exposure[2] to chlorpyrifos in rats.

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
Guo-Ross et al. (2007)	oral gavage *(corn oil)*	PND 1-4	4 hrs after exposure	1 mg/kg/day	25% *	brain ChE
				1.5 mg/kg/day	45%	
Betancourt and Carr (2004)	oral gavage *(corn oil)*	PND 1-3	24 hrs after exposure (PND 4)	1.5 mg/kg/day	27% *	forebrain ChE
				3 mg/kg/day	45% *	
Song et al. (1997)	s.c. injection *(DMSO)*	PND 1-4	24 hrs after exposure (PND 5)	1 mg/kg/day	24% *	brainstem ChE
Tang et al. (1999)	oral gavage *(corn oil)*	every other day PND 1-5	24 hrs after exposure (PND 6)	3 mg/kg/day	38% *	brain (excluding cerebellum) ChE
Carr et al. (2001)	oral gavage *(corn oil)*	every other day PND 1-5	24 hrs after exposure (PND 6)	3 mg/kg/day	74% **	forebrain ChE
					70% **	hindbrain ChE
					20% **	serum ChE
					39% **	lung ChE
Richardson and Chambers (2005)	oral gavage *(corn oil)*	PND 1-6	6 hrs after exposure (PND 6)	1.5 mg/kg/day	49%	brain (excluding cerebellum and medulla-pons) ChE
Betancourt and Carr (2004)	oral gavage *(corn oil)*	PND 1-6	24 hrs after exposure (PND 7)	1.5 mg/kg/day	28% *	forebrain ChE
				3 mg/kg/day	43% *	
Guo-Ross et al. (2007)	oral gavage *(corn oil)*	PND 1-8	4 hrs after exposure	1 mg/kg/day	N/A	brain ChE
				1 mg/kg PND 1-4 & 2 mg/kg PND 6-8	47% *	
				1.5 mg/kg PND 1-4 & 3 mg/kg PND 6-8	65% *	
Carr et al. (2001)	oral gavage *(corn oil)*	every other day PND 1-9	24 hrs after exposure (PND 10)	3 mg/kg/day	18% **	forebrain ChE
					39% **	hindbrain ChE
					22% **	serum ChE
					32% **	diaphragm ChE
					45% **	heart ChE
					33% **	lung ChE
					29% **	skeletal muscle ChE

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
Betancourt and Carr (2004)	oral gavage *(corn oil)*	PND1-11	24 hrs after exposure (PND 12)	1.5	None	Forebrain ChE
				3	20%	
Richardson and Chambers (2005)	oral gavage *(corn oil)*	PND 1- 12	12 hrs after exposure	1.5 mg/kg/day	43%	brain (excluding cerebellum and medulla-pons) ChE
Tang et al. (1999)	oral gavage *(corn oil)*	every other day PND 1-13	24 hrs after exposure (PND 14)	3 mg/kg/day	37% *	brain (excluding cerebellum) ChE
Carr et al. (2001)	oral gavage *(corn oil)*	every other day PND 1-15	24 hrs after exposure (PND 16)	3 mg/kg/day	30% **	forebrain ChE
					23% **	hindbrain ChE
					45% **	diaphragm ChE
					55% **	heart ChE
					53% **	lung ChE
					20% **	skeletal muscle ChE
		every other day PND 1-19	24 hrs after exposure (PND 20)		35% **	forebrain ChE
					26% **	hindbrain ChE
					55%**	heart ChE
					47% **	lung ChE
Tang et al. (1999)	oral gavage *(corn oil)*	every other day PND 1-21	24 hrs after exposure (PND 22)	3 mg/kg/day	29% *	brain (excluding cerebellum) ChE
Richardson and Chambers (2005)	oral gavage *(corn oil)*	PND 1-21	24 hrs after exposure (PND 22)	1.5 mg/kg/day	36%	brain (excluding cerebellum and medulla-pons) ChE
Liu et al. (1999)	s.c. injection *(peanut oil)*	PND 7-13	24 hrs after exposure (PND 14)	5 mg/kg/day	64% (p value N/A)	cortex ChE
					74% (p value N/A)	striatum ChE
				10 mg/kg/day	N/A	cortex ChE
					N/A	striatum ChE
Jett et al. (2001)	s.c. injection *(peanut oil)*	PNDs 7, 11, 15	24 hrs after exposure	0.3 mg/kg/day	N/A	cerebellum ChE
					N/A	cortex ChE
					N/A	hippocampus ChE
				7 mg/kg/day	N/A	cerebellum ChE
					N/A	cortex ChE
					N/A	hippocampus ChE
Liu et al. (1999)	s.c. injection *(peanut oil)*	PND 7-20	24 hrs after exposure (PND 21)	5 mg/kg/day	60% (p value N/A)	cortex ChE
					68% (p value N/A)	striatum ChE
				10 mg/kg/day	N/A	cortex ChE
					N/A	striatum ChE
Zheng et al. (2000)	oral gavage *(peanut oil)*	PND 7-20	4 hrs after exposure	0.15 mg/kg/day	20%	frontal cortex ChE
					13%	RBC ChE
					12%	plasma ChE
				0.45 mg/kg/day	7.5%	frontal cortex ChE
					25%	RBC ChE
					20%	plasma ChE

Study	Route *(vehicle)*	Time of exposure	Time of measurement	Dose	Inhibition[3]	Compartment[4]
				0.75 mg/kg/day	5%	frontal cortex ChE
					29%	RBC ChE
					24%	plasma ChE
				1.5 mg/kg/day	42% *	frontal cortex ChE
					57%	RBC ChE
					59%	plasma ChE
				4.5 mg/kg/day	76%	frontal cortex ChE
					89%	RBC ChE
					77%	plasma ChE
				7.5 mg/kg/day	85%	frontal cortex ChE
					97%	RBC ChE
					86%	plasma ChE
				15 mg/kg/day	N/A	frontal cortex ChE
					N/A	RBC ChE
					N/A	plasma ChE
Song et al. (1997)	s.c. injection *(DMSO)*	PND 11-14	24 hrs after exposure (PND 15)	5 mg/kg/day	66% *	brainstem ChE
Jett et al. (2001)	s.c. injection *(peanut oil)*	PNDs 22 & 26	PND 28	0.3 mg/kg/day	N/A	cerebellum ChE
					N/A	cortex ChE
					N/A	hippocampus ChE
				7 mg/kg/day	N/A	cerebellum ChE
					N/A	cortex ChE
					N/A	hippocampus ChE

[1] For many of the studies, inhibition levels were inferred from graphs.
[2] Data only provided for studies in which pups were directly dosed; data from lactational transfer studies were not included.
[3] Levels are % inhibition, compared to controls.
[4] Compartments are given as described by the study author(s).

AChE = acetylcholinesterase
ChE = cholinesterase, reported as either total (acetyl- and butyryl) or not specified
DMSO = dimethyl sulfoxide
F = female
GD = gestational day
i.p. = intraperitoneal
M = male
N/A = not available
PND = postnatal day
RBC = red blood cell
s.c. = subcutaneous

* $p<0.05$
** $p<0.01$

6.0 Benchmark Dose Analysis for Cholinesterase Inhibition

Numerous scientific peer review panels over the last decade have supported the Agency's application of the BMD approach as an improvement over the historically applied approach of using no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect-levels (LOAELs) and as a scientifically supportable method for deriving Points of Departure (PoDs) in human health risk assessment. The NOAEL/LOAEL approach does not account for the variability and uncertainty in the experimental results, which are due to characteristics of the study design, such as dose selection, dose spacing, and sample size. With the BMD approach, all the dose response data are used to derive a PoD. As a preliminary analysis, the Agency has conducted BMD modeling on selected studies described previously in this Appendix. These studies were selected based on the availability of at least two treatment groups and age/lifestage of animals tested. Selected studies include gestational exposures to the dam and acute and repeated exposures to post-natal pups of ages spanning PND1 up to PND 20.

In brief, the Agency has used a decreasing exponential dose-response model similar to that used for the OP and *N*-methyl carbamate cumulative risk assessments and previously reviewed by the FIFRA SAP on several occasions (FIFRA SAP 2001, 2002, 2005a, 2005b). Consistent with risk assessment on other OP and *N*-methyl carbamate compounds, the Agency has used a benchmark response level of 10% and has thus calculated BMD_{10}s and $BMDL_{10}$s. These values (the central estimate and lower confidence bound, respectively) represent the estimate dose where AChE is inhibited by 10% compared to background. Extensive analyses conducted as part of the OP cumulative risk assessment (USEPA, 2002) have demonstrated that 10% is a level that can be reliably measured in the majority of rat toxicity studies, and is generally at or near the limit of sensitivity for discerning a statistically significant decrease in AChE activity across the brain compartment and is a response level close to the background AChE level.

As shown in Table 8, the preliminary BMD analysis has included brain, RBC, and heart ChE inhibition data. These BMD estimates are discussed in detail in the chlorpyrifos Hazard and Dose Response Characterization document.

Table 8. Summary of Benchmark Dose Analyses for Acute and Repeat Studies

Reference	Age	Brain (mg/kg/day)		RBC or Heart (mg/kg/day)	
		BMD_{10}	$BMDL_{10}$	BMD_{10}	$BMDL_{10}$
Acute/Single Dose Studies					
Betancourt and Carr (2004)	PND 1	0.12	NR	NA	
Zheng et al. (2000)	PND 7 (male)	0.41[A]	0.25[A]	0.54	0.34
Timchalk et al. (2006)	PND 12	0.64	0.54	0.25	0.08
Timchalk et al. (2006)	PND 17	NA		1.12	0.48
Moser et al. (2006)	PND 17	0.87	0.67	NA	
Zheng et al. (2000)	Adult (male)	5.83[A]	3.09[A]	0.61	0.48
Repeat Dose Studies					
Betancourt and Carr (2004)	PND 1-3	0.44	0.22	NA	
Zheng et al. (2000)	PND 7-20 male	1.4[A]	0.95[A]	0.21	0.14
Zheng et al. (2000)	Adult, 14 days male	0.83[A]	0.40[A]	0.20	0.095
Betancourt and Carr (2004)	PND 1-6	0.41	0.198	NA	
2006 Cumulative RA	Repeated >21 days, Adult Female (non-pregnant)	1.48	1.26	NA	
Dow (Hoberman et al. 1998a,b, MRID44556901); Maurissen, 2000	Dam, GD6-20	0.65	0.54	0.06	0.03

Reference	Age	Brain (mg/kg/day)		RBC or Heart (mg/kg/day)	
		BMD_{10}	$BMDL_{10}$	BMD_{10}	$BMDL_{10}$
Dow (Mattsson et al. 1998 44648101); Mattsson, 2000	Dam, GD6-20	Hindbrain 1.10 Forebrain 1.17	Hindbrain 0.81 Forebrain 0.98	RBC 0.14 Heart 0.16	RBC 0.08 Heart 0.12
Dow (Mattsson et al. 1998 44648101); Mattsson, 2000	Dam, LD 1	Hindbrain 0.96 Forebrain 1.11	Hindbrain 0.55 Forebrain 0.77	RBC 0.079 Heart 0.109	RBC 0.0498 Heart 0.056

NA= Not applicable; [A]data for brain may be unreliable (see text)

7.0 Summary of Quantitative Differences in Cholinesterase Inhibition

Several studies published in the peer-reviewed literature and previously discussed have evaluated the differential sensitivities between adults and young animals following *in utero* and/or postnatal exposure to chlorpyrifos. Table 9 presents a brief comparison of the responses to chlorpyrifos between different age groups.

Table 9. Relative Responses of male, female, juvenile, neonatal and fetal rats to chlorpyrifos

Endpoint	Response	Comments
ChEI - BMD_{10} (Zheng et al., 2000) **RBC** **Plasma**	PND 7 neonate –0.54 mg/kg; adults-0.61 mg/kg PND 7 neonate-0.178 mg/kg; adults –0.629 mg/kg	Sensitivity: Neonate 1.1-fold >adult Neonate 3.5-fold >adult
ChEI - BMD_{10}: males Zheng et al. 2000 pup vs. Mendrala and Brzak (1998) adult **Plasma**	PND 7 neonate-0.178 mg/kg (4 hr); adults –0.571 mg/kg (3 hr); 0.287 mg/kg (6 hr)	Sensitivity: Neonate 1.6-3.2-fold >adult
ChEI - BMD_{10} 14 Days (Zheng et al. 2000) **Brain** **RBC** **Plasma**	PND 7 neonate-1.4 mg/kg; adults 0.827 mg/kg PND 7 neonate –0.209 mg/kg; adults-0.199 mg/kg PND 7 neonate-0.2 mg/kg; adults –0.19 mg/kg	Sensitivity: Adult 1.7-fold > neonate none none
ChEI - male and female rats (Mendrala and Brzak, 1998; Lassiter et al. 1998; Moser et al. 1998; Zheng et. al., 2000)	Male rats: slight (about 15%) brain ChEI at 10 mg/kg (2 studies); Male rats: 40% brain ChEI at 20 mg/kg; Female rats: 70% brain ChEI at 20 mg/kg; Female pregnant rats: 50% brain ChEI at 10 mg/kg	Pregnant female rats about 2-fold more sensitive than male rats to brain ChEI
Acute neurotoxicity (Moser et al. 1998)	PND17 juvenile- neurotoxicity at 20mg/kg; adult females-neurotoxicity at 50 mg/kg	Juvenile 2.5-fold more sensitive than adult
Muscarinic down regulation-acute dose (17 day juveniles) (Moser et al. 1998)	PND17 juvenile-down regulation at 15 mg/kg; adult females-down regulation at 80 mg/kg	Juvenile 5.3-fold adult (at the respective doses, down regulation was more extensive in young rats)

8.0. Discussion of ChE data

Chlorpyrifos, like other OPs, binds to and phosphorylates the enzyme, AChE, in both the central (brain) and peripheral nervous systems leading to accumulation of acetylcholine and, ultimately, to clinical signs of toxicity. This mode of action, in which AChE inhibition leads to neurotoxicity, has been well described (Mileson et al, 1998). In 2000, the Agency concluded for chlorpyrifos that inhibition of ChE was the most sensitive effect in all of the animal species evaluated and in humans, regardless of exposure duration. For the current analysis, the Agency has reviewed the studies submitted for registration as well as searched the public literature for studies in which pregnant animals and/or juvenile animals were exposed to chlorpyrifos. ChE inhibition is most commonly reported for the blood (plasma and RBC) and brain (whole or subsections), although a few studies have evaluated inhibition in peripheral tissues such as the heart, diaphragm, or lung.

Tables 10-12 provide summary information from AChE studies in gestational and post-natal studies in rats. The information provided here focuses on effects at or near a dose of 1-1.5 mg/kg. This dose has been used by numerous investigators evaluating both AChE inhibition and other toxicities. As such this dose provides a comparison point for comparing among studies, different toxicities, duration of exposure, ages, lifestages, and methods of administration. Comparisons across different studies need to be made with care as timing of sampling varies among studies which impacts results.

8.1. Gestational exposure

In gestational studies with chlorpyrifos, AChE activity is generally inhibited more in dams than in the fetus (Table 10). A similar pattern has been seen for many other OPs (USEPA, 2006, Attachment 1 to the Issue Paper). As such, it would appear that the fetus may be protected by the dam. However, rat fetal brain ChE activity increases 4 times from GD14 to GD18 and that activity increases another 3 times from GD18 to PND1. According to Lassiter et al (1998a), “new synthesis of uninhibited cholinesterase molecules may dilute the inhibited molecules such that the fetal brain cholinesterase activity recovers more quickly than the maternal brain.” Therefore, at a given time after exposure, cholinesterase may appear less inhibited by chlorpyrifos in the fetus compared to adults because the fetus recovers more quickly by rapidly synthesizing new brain cholinesterase. Further support for Lassiter’s comments are found in toxicokinetic (TK) studies. Following gestational exposure to the dam, Hunter et al. (1999) found that levels of TCP in the fetal brain were 2-3 times higher than in the maternal brain. Additional data are found in Mattsson et al (1998, 2000) who showed that chlorpyrifos levels were 2-fold higher in maternal blood than fetal blood but TCP levels were similar. Thus, when the dam is exposed to chlorpyrifos, the fetus is as well--likely at similar levels. As such, although the AChE data consistently shows more inhibition in the dam compared with the fetus, the fetus may not actually be protected by the dam. Therefore, AChE data in fetuses from repeated dosing gestational studies may not accurately reflect potential fetal toxicity at a particular dose.

Table 10. Summary of repeated studies evaluating gestational exposure to maternal rats and fetuses.

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Fetal inhibition	Maternal inhibition	Compartment
Mattsson et al. (1998, 2000)	oral gavage *(corn oil)*	GD6-20	4 hrs	1 mg/kg/day	8% (NS)	10%/7% (p<0.02)	forebrain
					0%	12%/7% (NS)	hindbrain
					5% (NS)	87%/85% (p<0.02)	RBC
					4% (NS)	77%/60% (p<0.02)	plasma
					0%	49%/50% (p<0.02)	heart
Hoberman et al. 1998a,b, Maurissen et al (2000)	oral gavage (corn oil)	GD6-20	4-5 hrs	1 mg/kg/day	N/A	68.9%	plasma
					N/A	84.4%	RBC
					N/A	17.9%	brain
Mattsson et al. 1998, (2000)	oral gavage *(corn oil)*	GD6-PND1	2 hrs	1 mg/kg/day	5% (NS)	6% (NS)	forebrain
					0%	6% (NS)	hindbrain
					0%	90% (p<0.02)	RBC
					5% (NS)	80% (p<0.02)	plasma
					2% (NS)	40% (p<0.02)	heart
Qiao et al. (2002)	s.c. injection *(DMSO)*	GD 17-20	GD 21	1 mg/kg/day	3% (NS)	brainstem	N/A
					6% (NS)	forebrain	N/A

8.2. Post-natal, acute exposures

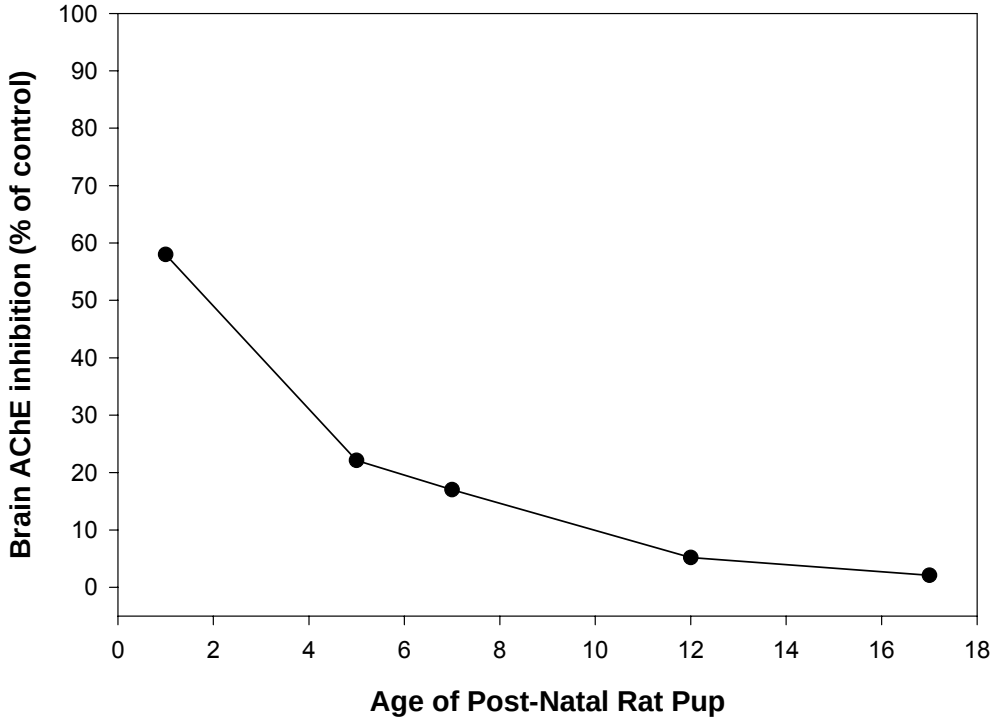
In post-natal studies where pups are directly exposed, the degree of ChE inhibition is clearly age dependant following single exposures (Table 11, Figure 1). In general, blood and peripheral measures are more inhibited at the same dose compared with brain measures. As mentioned in the Issue Paper, newborn and juvenile rats are more sensitive to AChE inhibition caused by chlorpyrifos than adult rodents, not because of a difference in the affinity of chlorpyrifos oxon to AChE, but because maturation of detoxification enzymes (Iyer, 2001). This ontogeny (and resulting reduced sensitivity) is evident in Figure 1 where the degree of brain AChE inhibition decreases with the age of the post-natal pup.

Table 11. Summary of acute studies evaluating post-natal exposure to juvenile rats.

Study	Route (vehicle)	Age	Time of measurement post-dosing[a]	Dose	Inhibition	Compartment
Dam et al. (2000)	s.c. injection (DMSO)	PND 1	2 hrs	1 mg/kg	70% (M); 25% (F)	brainstem
					80% (M); 10% (F)	cerebellum
					60% (M); 35% (F)	forebrain
Betancourt and Carr (2004)	oral gavage (corn oil)	PND 1	12 hrs	1.5 mg/kg	58%	forebrain
Timchalk et al. (2006)	oral gavage	PND 5	3 hrs	1 mg/kg	22.1%	brain
					45.7%	RBC
					62.1%	plasma
Zheng et al, 2000	oral gavage (peanut oil)	PND 7	4 hrs	1.5 mg/kg	17%	frontal cortex
					32%	RBC
					51%	plasma
Timchalk et al. (2006)	or al gavage	PND 12	6 hrs	1 mg/kg	5.2%	brain
					27.0%	RBC
					33.4%	plasma
Timchalk et al. (2006)	oral gavage	PND 17	24 hrs	1 mg/kg	2.1%	brain
					15%	RBC
					21.9%	plasma
Moser et al. 2006	oral gavage (corn oil)	PND17	4.5 hrs	0.5 mg/kg	0%	brain
				2 mg/kg	10%	
				0.5 mg/kg	10%	whole blood
				2 mg/kg	40%	

a. Reported time of peak effect

Figure 1. Plot of brain AChE inhibition in post-natal pups following a single dose of 1 mg/kg



8.3. Pregnant Dams and Fetuses

Table 12 and Figure 2 summarize information from repeated dosing studies in post-natal pups using a dose of 1-1.5 mg/kg/day as a point of comparison. Repeated dosing studies show similar degrees of brain AChE inhibition independent of duration of exposure. For example, Guo-Ross et al (2007) and Richard and Chambers (2005) each measured similar amounts of brain AChE inhibition but Guo-Ross et al (2007) dosed pups with only 4 exposures whereas Richard and Chambers (2005) used 6 exposures. The pattern of similar degrees of AChE inhibition across repeated dosing post-natal studies likely reflects the rapid nature of AChE recovery observed by multiple investigators (Chakraborti *et al.,* 1993; Moser and Padilla, 1998; Pope et al., 1991; Pope and Liu, 1997). This pattern is less evident at higher doses where AChE inhibition has reached >70-80% and/or where metabolic processes may be saturated (Table 7).

One exception to this is the PND1-11 group in Betancourt and Carr (2004) where no significant brain AChE inhibition was reported. When evaluating the results within the Betancourt and Carr (2004) study, there is a decrease in inhibition following repeated dosing studies from PND1-3, 1-6, and 1-11 suggesting that as the pups mature, they become less sensitive. A similar but less pronounced trend was observed by Richards and Chambers (2005) who showed that PND1-6 and 1-12 dosing resulted in similar degrees of inhibition. In the PND1-21 group, a somewhat lower amount of

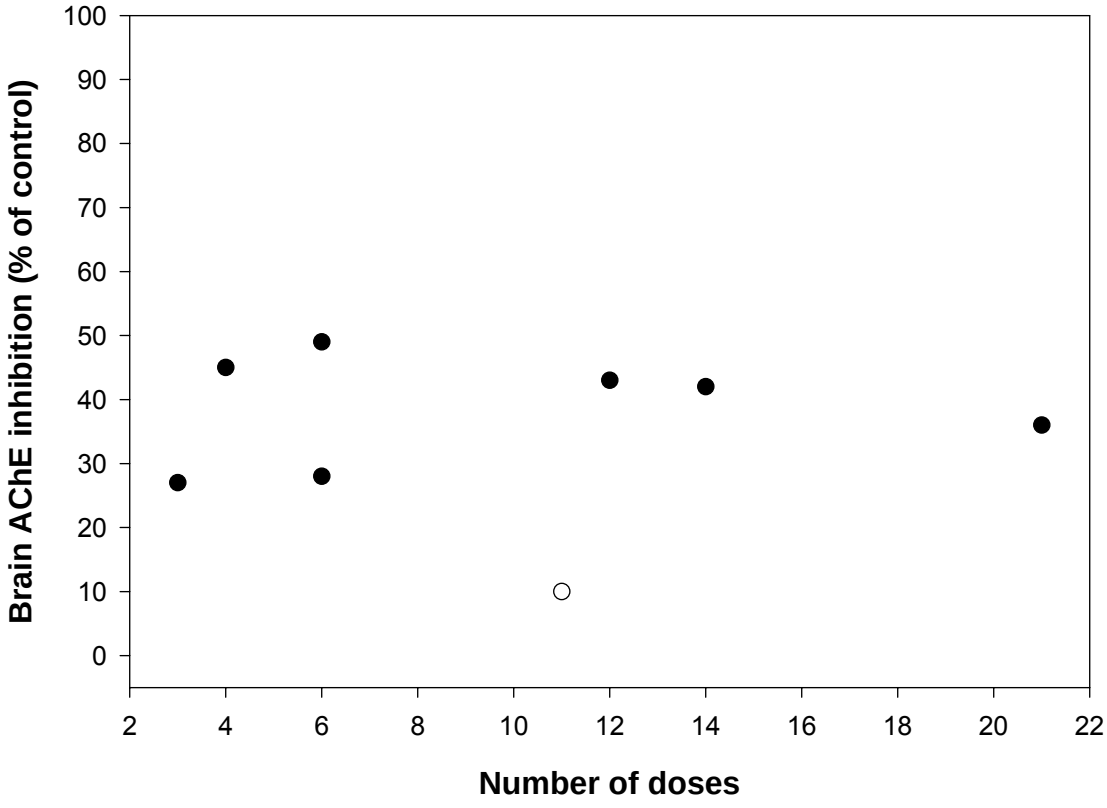
brain AChE inhibition was observed (36%) compared with the PND1-6 and 1-12 groups. There are potential explanations for this. First, the results of Richards and Chambers (2005) may be explained based on the timing of measurement, PND1-6 animals were measured at 6 hours post-dosing but the PND 1-21 was measured 24 hours post-dosing. Alternatively, the reduced brain AChE inhibition could have resulted from maturation of detoxification pathways resulting in decreased inhibition.

It is notable that the trend shown in Figure 2 is distinctly different from results in adult studies for most OPs. Typically in adult rats, AChE inhibition increases with repeated exposures. In other words, at a common dose level, more inhibition is observed after repeated exposures compared with a single exposure.

Table 12. Summary of repeated studies evaluating post-natal exposure to juvenile rats.

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Inhibition	Compartment
Guo-Ross et al. (2007)	oral gavage (corn oil)	PND 1-4	4 hrs	1 mg/kg/day	25%	brain
				1.5 mg/kg/day	45%	
Betancourt and Carr (2004)	oral gavage	PND 1-3	24 hrs	1.5 mg/kg/day	27%	forebrain
Song et al. (1997)	s.c. injection (DMSO)	PND 1-4	24 hrs	1 mg/kg/day	24%	brainstem
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-6	6 hrs	1.5 mg/kg/day	49%	brain (excluding cerebellum and medulla-pons)
Betancourt and Carr (2004)	oral gavage	PND 1-6	24 hrs	1.5 mg/kg/day	28%	forebrain
Betancourt and Carr (2004)	oral gavage	PND1-11	24 hrs	1.5 mg/kg/day	None	forebrain
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1- 12	12 hrs	1.5 mg/kg/day	43%	brain (excluding cerebellum and medulla-pons)
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-21	24 hrs	1.5 mg/kg/day	36%	brain (excluding cerebellum and medulla-pons)
Zheng et al (2000)	oral gavage (peanut oil)	PND7-20	4 hrs	1.5 mg/kg/day	42%	frontal cortex
					57%	RBC
					59%	plasma

Figure 2. Plot of brain AChE inhibition in post-natal pups following repeated dosing at 1.5 mg/kg



8.4. Method of administration

AChE studies available for chlorpyrifos use a variety of methods of administration. The two most common are oral gavage and subcutaneous injection, particularly with DMSO. Some have suggested that the TK properties of a particular chemical may vary by method of administration, thereby impacting the amount of AChE inhibition observed in a particular study. However, the Agency's analysis suggests that the inhibition levels may be more similar than previously believed.

In general, study designs in gestational studies vary widely among laboratories with regard to doses used, number of repeated doses, and gestational days of dosing which makes comparing the results problematic. Chanda and Pope (1996) exposed dams from GD 12-19 via subcutaneous injection with peanut oil and showed 75% brain inhibition 24 hours after the last dose in the dams at a dose of 6.25 mg/kg/day. Hunter et al (1999) and Lassiter et al (1998a) exposed dams using corn oil gavage from GD14-18 at 7 mg/kg/day

and observed 68% and 69% brain AChE inhibition 24 hours after the last dose, respectively. The gestational days of dosing differs between the studies and the number of doses differs between the studies--8 and 5 for Chanda and Pope (1996) and the EPA studies (Hunter et al, 1999; Lassiter et al, 1998a), respectively. Even when considering the differences in study designs, there is notable similarity in the amount of measured brain AChE inhibition at 24 hours after the last dose in the studies using subcutaneous injection and oral gavage—75% and 68-69% (Chanda and Pope, 1996; Hunter et al, 1999; Lassiter et al, 1998a, respectively).

Comparison of post-natal studies show that brain AChE inhibition at similar dose levels (e.g., Tables 11 and 12) yields remarkably similar results in young pups (ages PND1-5). For example following an acute dose of 1 or 1.5 mg/kg/day in PND1 pups, 60% and 58% forebrain AChE inhibition were noted from subcutaneous injection with DMSO and corn oil gavage, respectively (Dam et al, 2000; Betacourt and Carr, 2004). Following exposure at 1 mg/kg/day from PND1 to PND4, 24% and 25% brain AChE inhibition were noted from subcutaneous injection with DMSO and corn oil gavage, respectively (Song et al, 1997; Guo-Ross et al, 2007). The time measurements of these studies were at 24 hour and 4 hours post-dosing for subcutaneous injection and gavage, respectively. The Agency also notes that preliminary (not yet replicated or published by the authors) data by Carr and Narr presented at SOT (2008) showed striking similarity in the time course and amount of brain AChE inhibition in PND10 pups exposed at 5 mg/kg from subcutaneous injection with DMSO and corn oil gavage. Moreover, the amount of brain AChE (25-28%) in the Carr poster is similar to that PND11 pups exposed at the same dose from Dam et al (2000) who used subcutaneous injection (15-30% brain stem, but 15-35% for brain) at 4 hours post-dosing.

A recent study by Marty et al (2007) provides TK data which supports findings of post-natal AChE studies mentioned above. Specifically, Marty et al (2007) compared methods of administration for PND5 pups exposed to 1 mg/kg/day chlorpyrifos via corn oil gavage, subcutaneous injection with DMSO, and oral exposure in milk. Across the three methods of administration, Marty et al (2007) showed relatively small (2-fold or less) differences in: 1) AUC for chlorpyrifos and TCP; 2) ½ lives for TCP; and 3) similar time to peak effect for chlorpyrifos and TCP. Based on the findings of Marty et al (2007), there appear to be only small differences in TK characteristics in PND5 pups exposed via corn oil gavage, subcutaneous injection with DMSO, and exposure in milk.

The Agency has concluded for young pups, at least up to PND 5 in rat, that administration via the oral route and subcutaneous injection provide remarkably similar results and that post-natal studies up to PND 5 in either route are relevant for risk assessment. Less data are available to compare routes/methods of administration for older pups and no comparative TK data are available for gestational exposures. As more studies are available in the future,

the Agency may, if appropriate, extend this conclusion to include older pups. The lack of comparative PK for oral gavage and subcutaneous injection in pregnant dams and fetuses in considered an important data gap in quantitatively evaluating dose response data in subcutaneous injection studies (as discussed in Appendix C). However, the Agency can not discount the findings of subcutaneous injection gestational studies at this time.

8.5. Preliminary conclusions

Numerous AChE studies are available in different lifestages and ages in rats. These studies vary widely by the level and number of doses used, availability of time course information, and method of administration. The Agency has preliminarily concluded the following

- Repeated dosing gestational studies which show less fetal brain AChE inhibition compared with the dam may not reflect actual toxicity to the pup. This conclusion is based, in large part, on TK data comparing blood and brain levels of chlorpyrifos and/or its metabolites in fetal and dam tissues.
- Following acute post-natal exposure studies, there is an age-dependant sensitivity that decreases as the pups mature.
- When considering the repeated dosing post-natal studies across laboratories, there is little variability with respect to degree of brain AChE inhibition across different durations of exposure. Within a laboratory, however, decreases in sensitivity have been observed with longer duration of exposure.

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Attachment A: Summary of Registrant Toxicology Studies for Chlorpyrifos

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
Subchronic Toxicity			
82-1(a)	Subchronic Feeding in Rats (90 days) MRID #: 40436406 Makhteshim-Agan; Crown et al. 1985 Core Grade: acceptable guideline	0, 0.025, 0.5, or 10 (0, 0.5, 10 or 200 ppm)	**95.5% a.i. chlorpyrifos** NOAEL ChEI: none for plasma ChEI due to reductions in male plasma enzymes at 0.025 LOAEL ChEI: 0.025 (significant 22%↓ in plasma ChE activity that was dose-related) NOAEL (systemic): 0.5 LOAEL (systemic): 10 Effects: decreased weight gain and slight decreases in packed cell volume, red cells and hemoglobin Note: Female ChEI data is unreliable due to a possible reporting error. RBC and brain ChE activity were not measured.
82-1(a)	Subchronic Feeding in Rats (90 days) MRID #: 40952801 Szabo et al. 1988 Core Grade: acceptable guideline	0, 0.1, 1, 5 or 15	**95.7 - 98.5% chlorpyrifos** NOAEL: 0.1 (plasma and RBC ChEI) LOAEL: 1 (significant plasma and RBC ChEI in both sexes) Effects: increased organ weights (brain and heart), and reduced weight gain at 15 mg/kg/day and increased adrenal gland vacuolation and significant brain ChEI in both sexes 5 and 15 mg/kg/day.
82-1(b)	Subchronic Oral (capsule) in Beagle Dogs MRID #: 42172801 Barker 1989 Core Grade: acceptable guideline	0, 0.01, 0.22, or 5	**95.8% chlorpyrifos** NOAEL: 0.01 LOAEL: 0.22 (significant 33-67% ↓ plasma and 24-46% ↓ RBC ChEI) Effects: Brain ChEI (46% ↓) occurred at 5 mg/kg/day. Comments: At 0.01 mg/kg/day, plasma ChEI noted in females (significant 20-24% at week 6, and non-significant 24% at week 12) and males (15% at week 13) that was not considered of sufficient magnitude and consistency to be biologically and toxicologically meaningful.
	Subchronic Oral feeding in Beagle Dogs MRID #: 45466501 2001 Marable, B.; Baker, P.; Stebbins, K.; et. al. (2001) Core Grade: acceptable non-guideline for RBC and brain ChE only	0, 0.5, 1 or 2 mg/kg	**97.6% chlorpyrifos** NOAEL: < 0.5 LOAEL: 0.5 (significant 41-56% ↓ RBC ChE for females and males, respectively) Effects: No significant Brain ChEI at 2 mg/kg/day.

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
82-2	21-Day Dermal Toxicity Study in Rats and 4-day Dermal Probe Study MRID #: 40972801 Calhoun and Johnson 1988 Core Grade: acceptable guideline	0, 0.1, 0.5, 1 or 5 (21 day study) 0, 1, 10, 100 or 500 (4-day dermal probe study)	**100% chlorpyrifos** NOAEL: 5 (plasma and RBC ChEI) LOAEL: 10 (45% plasma and 16% RBC ChEI following 4 days of exposure) NOAEL (systemic): not identified LOAEL (systemic): not identified (>5) Effects: Slight erythema in 2/4 females at 1 and 10 mg/kg/day, respectively.
82-4	Subchronic Inhalation in Rats (90 days) (nose only) MRID #: 40013901 & 40166501 Corley et al. 1986a,b Core Grade: acceptable guideline	0, 5.2, 10.3 or 20.6 ppb (0, 72, 143 or 287 µg/m^3) (maximum dose equivalent to 0.044-0.082 mg/kg/day)	**100% chlorpyrifos** NOAEL: not identified (ChEI and systemic) LOAEL: not identified at highest attainable vapor concentration (>20.6 ppb or 287 µg/m^3) (ChEI and systemic)
82-4	Subchronic Inhalation in Rats (90 days) (nose only) MRID #: 40908401 Makhteshim-Agan; Newton 1988 Core Grade: acceptable guideline	0, 5, 10 or 20 ppb (0, 70, 143 or 287 µg/m^3) (equivalent to 0, 0.024, 0.048 or 0.097 mg/kg/day, respectively)	**95% chlorpyrifos** NOAEL: not identified (ChEI and systemic) LOAEL: not identified at highest attainable vapor concentration (>20 ppb) (ChEI and systemic)
Chronic Toxicity/Carcinogenicity			
83-1& 2	Chronic feeding/ carcinogenicity study in F344 rats (2 yrs) MRID # 42172802 Crown et al. 1990 Core Grade: acceptable guideline	Males: 0, 0.0132, 0.33 or 6.99 and Females: 0, 0.0146, 0.365 or 7.78 (0, 0.2, 5 or 100 ppm)	**96.1% chlorpyrifos** NOAEL:0.0132 LOAEL: 0.33 (significant 15-51% plasma ChEI in both sexes, 19-31% RBC ChEI at 104 weeks vs. controls and 11-17% RBC ChEI vs. vehicle controls) NOAEL (systemic):0.33 LOAEL (systemic): 6.99 Effects: decreased body weights in males and females, and cataracts, and diffuse retinal atrophy in females. No evidence of carcinogenicity.

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
83-1 & 2	Chronic feeding/ carcinogenicity study in F344 rats (2 yrs) MRID # 40952802 Young and Grandjean 1988 Core Grade: acceptable guideline	0, 0.05, 0.1, 1 or 10	**Lorsban 98.5% chlorpyrifos** NOAEL: 0.1(plasma and brain ChEI) LOAEL: 1 (significant 39-86% plasma, 14-34% RBC and 5-9% brain ChEI) NOAEL (systemic): 1 LOAEL (systemic): 10 Effects: decreased body weight gain, red blood cells, hemoglobin, cholesterol, protein, and globulin, and increased platelets and specific gravity, increased adrenal gland weight, and fatty vacuolation of the zona fasciculata. No evidence of carcinogenicity.
83-1b	Chronic feeding study in beagle dogs (2 yrs) MRID # 00064933 & 00146519 McCollister et al. 1971, Kociba 1985 Core Grade: acceptable guideline	0, 0.01, 0.03, 0.1, 1 or 3	**97.2-98.8% chlorpyrifos** NOAEL: 0.01, 0.03, & 1 for plasma, RBC and brain ChEI, respectively LOAEL (plasma ChEI): 0.03 (mostly significant mean of 23-29% ↓ at 1 year and 10-24% ↓ at 2 years) LOAEL (RBC ChEI): can not be established due to data quality issues LOAEL (brain ChEI): 3 (19.4-20.8% ↓ at 2 yr) NOAEL (systemic): 1 LOAEL (systemic): 3 Effects: increased absolute and relative liver weights that could be an adaptive response
83-2	Chronic feeding study in CD-1 mice (2 yrs) MRID # 00054352 & 00142902 (Accession No. 242059) Warner et al. 1980 Core Grade: acceptable guideline	0, 0.5, 5 or 15 ppm (highest dose tested is 2.25 mg/kg/day)	**99.6% chlorpyrifos** LOAEL: 2.25 (90%↓plasma, and 50%↓ RBC ChE activity relative to controls after 1 week) NOAEL(systemic) = 2.25 LOAEL (systemic): none observed (>2.25) Effects: no systemic effects observed at highest dose tested (HDT). No treatment-related tumors. ChE only measured at 15 ppm (2.25 mg/kg/day) after 1 and 4 weeks.

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
83-2	Chronic feeding/ carcinogenicity study in CD-1 mice (78 weeks) MRID # 42534201 Gur 1992 Core Grade: acceptable guideline	Males: 0, 0.89, 8.84, 45.2 Females: 0, 0.938, 9.79, or 48.1 (0, 5, 50 or 250 ppm)	**95.5% chlorpyrifos** NOAEL: none for ChEI LOAEL: 0.89 males; 0.938 females (significant 45-51% plasma ChEI in both sexes) NOAEL (systemic): 8.84 males, 9.79 females (50 ppm) LOAEL (systemic): 48.1 females, 45.2 males (HDT; 250 ppm) Effects: decreased body weight gain and food consumption in males, decreased water consumption in females, increased incidences of keratitis and hepatocyte fatty vacuolation, and increased incidence of gross clinical findings (ocular opacity and hair loss) in both sexes. Brain ChE was inhibited at the high dose in both sexes. No evidence of carcinogenicity. Note: The validity of the RBC ChE assay is questionable.
Developmental Toxicity			
83-3a	Developmental Study in CD rats (gavage) MRID# 40436407 Makhteshim-Agan; Rubin et al. 1987a Core Grade: acceptable guideline	0, 0.5, 2.5 or 15 (gestation day 6-15)	**96.1% chlorpyrifos** Maternal NOAEL: none observed for plasma ChEI; 2.5 for systemic Maternal LOAEL: 0.5 (decreased plasma ChEI); 15 (systemic) based on decreased food consumption (only the first few days of dosing) and body weight during dosing. Developmental NOAEL: 2.5 Developmental LOAEL: 15 (HDT) based on an increase in post-implantation loss. Comments: RBC and brain ChE were not measured.
83-3a	Developmental Study in F344 rats (gavage) MRID# 00130400 Ouellette et al. 1983 Core Grade: acceptable guideline	0, 0.1, 3, or 15 (gestation day 6-15)	**96.6% chlorpyrifos** Maternal NOAEL: 0.1 (plasma and RBC ChEI) Maternal LOAEL: 3 (90.3% plasma and 74.3% RBC ChEI) Developmental NOAEL: none identified Developmental LOAEL: none identified (>15 highest dose tested, HDT)

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
83-3a	Developmental Study in CF-1 mice (gavage) MRID# 00095268 Deacon et al. 1979 Core Grade: Not acceptable guideline	0, 0.1, 1, 10, or 25 (gestation day 6-15)	**96.8% chlorpyrifos** Maternal NOAEL: 0.1 (plasma and RBC ChEI); 10 (systemic toxicity) Maternal LOAEL: 1 (plasma and RBC ChEI); 25 (systemic toxicity) based on decreased body weight, food and water consumption, and increased mortality. Developmental NOAEL: 1 (plasma and RBC ChEI); 10 for systemic toxicity Developmental LOAEL: 10 (plasma and RBC ChEI); 25 (systemic toxicity) based on minor skull variations, delayed ossification of skull bones and sternebrae and reduced fetal body length. Comments: Brain ChE not measured.
83-3 (b)	Developmental Study in New Zealand rabbits (gavage) MRID# 40436408 Makhteshim-Agan; Rubin et al. 1987b Core Grade: acceptable guideline	0, 1, 9, 81, or 140 (gestation day 7-19)	**96.1% chlorpyrifos** Maternal NOAEL: none observed for plasma ChEI; 81 for systemic toxicity Maternal LOAEL: 1 (plasma ChEI); 140 for systemic toxicity based on reduced food consumption, body weight loss, and apparent post-implantation loss. Developmental NOAEL (systemic): 81 Developmental LOAEL (systemic): 140 based on slightly decreased fetal weights and crown-rump lengths, and an increased incidence of unossified xiphisternum and/or 5th sternebra.
83-6	Developmental Neurotoxicity Study in Rats MRID: 44556901 Hoberman. 1998a,b Core Grade: unacceptable guideline, but upgradeable	0, 0.3, 1, or 5 (gestation day 6 through lactation day 11)	**99.8% chlorpyrifos** Maternal NOAEL: none observed for plasma or RBC ChEI Maternal LOAEL:≤0.3 (43%↓ plasma and 41%↓% RBC ChE activity relative to controls) Developmental NOAEL (systemic): can not be determined Developmental LOAEL(systemic):can not be determined Comments: at 1mg/kg/day significant treatment-related decrease in the measurement of the parietal cortex, supported by possible (although nonsignificant) alterations in the hippocampal gyrus, in the brain of female rats at postnatal day 66. Morphometric data for low-dose (0.3 mg/kg/day) female rats at postnatal day 66 have been requested.

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
NA	Cholinesterase and Metabolite Determination Study in Rats (Companion Study of the Developmental Neurotoxicity Study) MRID: 44648101 Mattsson et al. 1998 Core Grade: Acceptable Non-guideline	0, 0.3, 1, or 5 (gestation day 6 through lactation day 11)	**99.8% chlorpyrifos** Maternal Effects: Dams in the 0.3 mg/kg/day group exhibited a 52%↓ plasma and 39↓% RBC ChE activity relative to controls Developmental Effects: Pups in the 5 mg/kg/day group exhibited an 85%↓ plasma, 92↓% RBC, 82%↓ heart and 60%↓ brain ChE activity relative to controls Note: This is a pharmacokinetic study, and therefore, NOAELs and LOAELs were not identified
Reproductive Toxicity			
83-4	2-Generation Reproduction Toxicity in SD Rats MRID No: 41930301 Breslin et al. 1991 Core Grade: acceptable guideline	0, 0.1, 1, or 5 for 10 (F0) or 12 (F1) weeks prior to mating, through lactation and weaning	**97.8-98.5% chlorpyrifos** Parental NOAEL: 0.1 Parental LOAEL: 1 (significant 43-59% plasma, and 65-69% RBC ChEI at 1 mg/kg/day; and 48-49% brain ChEI and histological lesions of the adrenal gland at 5 mg/kg/day). Reproductive NOAEL: 1 Reproductive LOAEL: 5 (HDT) based on reduced pup weight and increased pup mortality in F1 generation only.
83-4	3-Generation Reproduction Toxicity in SD Rats MRID No: 00029064, 00064934 Thompson 1971 Core Grade: acceptable guideline	0, 0.03, 0.1,or 0.3 for first generation, and 0.1, 0.3 or 1 for second and third generation	Parental NOAEL: 0.1 Parental LOAEL: 0.3 (plasma and RBC ChEI) Reproductive NOAEL: >1 (HDT) Reproductive LOAEL: not identified
Neurotoxicity			
81-7	Delayed Neurotoxicity Study in Hens MRID No: 40510601 1987; Roberts et al. 1987 Core Grade: acceptable guideline	0, 50, 100 or 110	**96.8% chlorpyrifos** NOAEL: 110 (HDT); No delayed neurotoxicity
81-8	Acute Neurotoxicity Study in Rats MRID 42669101and 42943101 Wilmer et al. 1992 Core Grade: acceptable guideline	0, 10, 50 or 100	**98.2% chlorpyrifos** NOAEL (systemic): 10 LOAEL (systemic): 50 Effects: Decreased body weight, and motor activity and increased incidence of adverse clinical signs ChE activity not measured

GDLN	STUDY	DOSE (mg/kg/day)(1	RESULTS (mg/kg/day) (1)
NA	Acute Pharmacokinetic Study in Rats MRID 44648102 Mendrala and Brzak 1998 Core Grade: acceptable nonguideline	0.5, 1, 5, 10, 50, 100	**89.4-99.8% chlorpyrifos** NOAEL: 0.5 LOAEL: 1 (28-40% plasma ChEI at the peak time of inhibition, 3-6 hours post exposure) Other: significant brain ChEI at doses ≥10 Note: red blood cell ChE measurements were not collected.
82-8	13 Week Rat Neurotoxicity Study MRID 42929801 Shankar et al. 1993 Core Grade: acceptable guideline	0, 0.1, 1, 5, or 15	**98.2% chlorpyrifos** NOAEL (systemic): ≥15 LOAEL (systemic): none established Effects: Decreased motor activity and an increased incidence of urine incontinence in females. Note: This study did not measure ChE activity.
NA	Special Acute Neurotoxic Esterase (NTE) Rat Study MRID 44273901 Dittenber 1997 Core Grade: acceptable non-guideline	0, 1, 5, 10, 50 or 100	**98.1% chlorpyrifos** NOAEL: 1 [plasma ChE, and RBC and heart acetyl ChE] LOAEL: 5 (45% plasma ChEI; 17% RBC AChEI; and 19% heart AChEI). Effects: NTE was not inhibited at any dose. Note: ChE measurements were made 24 hours post exposure.
NA	Cognitive Rat Study MRID 44020901 Maurissen et al. 1996 Core Grade: Acceptable non guideline	0, 1, 3, or 10 for 5 days/week for 4 weeks	**98.1% chlorpyrifos** NOAEL: none observed (plasma and RBC ChE), LOAEL: 1 (68% plasma ChEI; 56% RBC ChEI and 8% brain ChEI). NOAEL (systemic): 1 (miosis) LOAEL (systemic): 3 (miosis)
83-6	Developmental Neurotoxicity Study in Rats MRID: 44556901 Hoberman. 1998a,b Core Grade: unacceptable guideline, but upgradeable	0, 0.3, 1, or 5 (gestation day 6 through lactation day 11)	**99.8% chlorpyrifos** Maternal NOAEL: none observed for plasma or RBC ChEI Maternal LOAEL: ≤0.3 (43%↓ plasma and 41%↓% RBC ChE activity relative to controls)

(1) Unless specified.
ChEI = Cholinesterase Inhibition
RBC = red blood cell
NOAEL = No Observable Adverse Effect Level
LOAEL = Lowest Observable Adverse Effect Level
NA= Not applicable